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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional application under 37 C.F.R. § 1.53(b))

Attorney Docket No.	98-1984
First Inventor or Application Identifier	L. S. Zagon
Title	Novel Nucleic Acid Molecules Encoding Opioid Growth Factor Receptors
Express Mail Label No.	EK087383601US

jc503 U.S. PTO 09/431842 11/02/99

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

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1. [X] * Fee Transmittal Form (*e.g.*, PTO/SB/17) *(Submit an original and a duplicate for fee processing*
2. [X] Specification [Total Pages 77]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings (*if filed*)
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) (35 U.S.C. 113) [Total Sheets 16]
4. Oath or Declaration [Total Sheets 2]
   a. [X] Unexecuted copy
   b. [ ] Copy from a prior application (37 C.F.R. §1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§1.63(d)(2) and 1.33(b)

NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. §1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. §1.28)

5. [ ] Microfiche Computer Program (Appendix)
6. Nucleotide and/or Amino Acid Sequence submission *(if applicable, all necessary)*
   a. [X] Computer Readable Copy
   b. [X] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement (when there is an assignee) [ ] Power of Attorney
9. [ ] English Translation Document (*if applicable*)
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [X] * Small Entity Statement(s) (PTO/SB/09-12) [ ] Statement filed in prior application, Status still proper and desired
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15. [ ] Other:

16. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below in a preliminary amendment*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part of prior application No.: Claims priority to 60/106,879 filed 11/03/98

Prior application information: Examiner _____ Group / Art Unit: _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION

Docket Number (Optional): 98-1984

Applicant, Patentee, or Identifier: I. S. Zagon

Application or Patent No.:

Filed or Issued: November 02, 1999

Title: Novel Nucleic Acid Molecules Encoding Opioid Growth Factor Receptors

I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below:

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NAME OF PERSON SIGNING: Thomas J. Monahan (Reg. No. 29,835)

TITLE IN ORGANIZATION OF PERSON SIGNING: Director, Intellectual Property Office

ADDRESS OF PERSON SIGNING: 113 Technology Center, University Park, PA 16802-7000

SIGNATURE _____ DATE: November 02, 1999

Practitioner's Docket No.: 98-1984 ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: I. S. Zagon

Serial No.: Group No.:

Filed: November 2, 1999 Examiner:

For: NOVEL NUCLEIC ACID MOLECULES ENCODING OPIOID GROWTH FACTOR RECEPTORS

Assistant Commissioner for Patents
Washington D.C. 20231

EXPRESS MAIL CERTIFICATE

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1 Small Entity Statement
1 unexecuted Declaration
77 pages of Specifications (including Sequence Listing)
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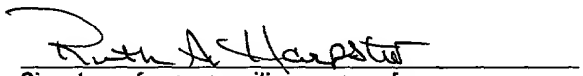
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NOVEL NUCLEIC ACID MOLECULES ENCODING OPIOID GROWTH FACTOR RECEPTORS

This application claims the priority of U.S. Provisional Application Serial No. 60/106,879, filed November 3, 1998.

FIELD OF THE INVENTION

This invention relates to novel nucleic acid molecules coding for opioid growth factor receptors. The invention further relates to the use of such nucleic acid molecules and compositions derived therefrom in modulating cell growth.

BACKGROUND OF THE INVENTION

Endogenous opioid peptides, first reported by Hughes and coworkers in 1975 (Hughes et al., *Nature* 258: 577-580, 1975), have been documented to be potent regulators of growth (Zagon and McLaughlin, *Opioid growth factor in the developing nervous system*, in: I.S. Zagon and P.J. McLaughlin (Eds.), *Receptors in the Developing Nervous System*, vol. 1, *Growth Factors and Hormones*, Chapman and Hall, London, UK, 1993, pp. 39-62), as well as neuromodulators (Akil et al., *Ann. Rev. Neurosci.* 7: 223-255, 1984). One native opioid peptide, [Met^5]-enkephalin, has been reported to be an inhibitory growth factor in development, cellular renewal, cancer, wound healing, and angiogenesis (Isayama, et al., *Brain Res.* 544: 79-85, 1991; McLaughlin, *Amer. J. Physiol.* 271: R122-129, 1996; Murgo, *J. Natl. Cancer Inst.* 75: 341-344, 1995; Steine-Martin, et al., *Life Sci.* 46: 91-98, 1990; . Villiger et al., *EMBO J* 11: 135-143, 1992; Zagon and

McLaughlin, 1993, *supra*; Zagon et al., *Amer. J. Physiol* 271: R780-R786, 1996; Zagon et al., *Brain Res.* 798: 254-260, 1998; Zagon et al., *Brain Res.* 803: 61-68, 1998. In view of these growth properties, [Met^5]-enkephalin has been termed opioid growth factor (OGF) (Zagon and McLaughlin, 1993, *supra*). OGF is an autocrine produced and secreted peptide that is not cell, tissue, or organ specific. While OGF exhibits activity at physiologically relevant concentrations, it does not elicit physical dependence, tolerance, and/or withdrawal. OGF displays a temporal and spatial distribution consistent with specific growth-related effects and is sensitive to opioid antagonist displacement. OGF has a direct, rapid, prolonged, stereospecific, receptor mediated, non-cytotoxic, and reversible influence on growth both in tissue culture and in prokaryotic and eukaryotic organisms. Blockade of the interaction between endogenous opioids and opioid receptors with compounds such as naltrexone (NTX) enhances growth (McLaughlin et al., *Physiol. Behav.* 62: 501-508, 1997; Zagon, et al., *Science* 221: 671-673, 1983; Zagon et al., *Science* 221: 1179-1180, 1983), suggesting that growth related opioid peptides such as OGF are tonically active. The molecular nature of OGF is well documented, and this peptide is encoded by the preproenkephalin gene (Gubler et al., *Nature* 295: 206-209, 1982; Noda et al. *Nature* 295: 202-206, 1982).

The receptor mediating the action of OGF shares certain pharmacological characteristics of classical opioid receptors, including the binding to opioids, stereospecificity, and naloxone-reversibility (Zagon et al., *Brain Res.* 551: 28-35, 1991; Zagon et al., *Brain*

Res. 482: 297-305, 1989). Thus, this receptor was originally - and tentatively - termed the zeta (ζ) opioid receptor. However, physiological, pharmacological, receptor binding assays and immunocytochemical localization experiments have revealed the novel nature of this receptor. In particular, the function (growth), tissue distribution (neural and non-neural), subcellular location (nuclear-associated), transient appearance during ontogeny, ligand specificity ([Met^5]-enkephalin), and competitive inhibition profile differ substantially from what is known about classical opioid receptors.

The present invention provides for the first time the molecular information of the receptor for OGF, in particular, the nucleotide and amino acid sequences of such receptor. Comparison of such sequences with those reported for the opioid receptor family shows no structural homology. In view of the pharmacological, biochemical, physiological and molecular differences, the present invention has termed the receptor tentatively identified as the zeta opioid receptor, the OGF receptor (OGFr).

SUMMARY OF THE INVENTION

One embodiment of the present invention is directed to isolated nucleic acid molecules coding for OGF receptors.

In a preferred embodiment, the present invention provides isolated nucleic acid molecules, SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13. Degenerate sequences, splice variant sequences, fragments, sequences having deletions, insertions or substitutions, as well as

homologs of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 are also contemplated by the present invention.

Another embodiment of the present invention is directed to isolated nucleic acid molecules, the complement sequences of which hybridize under stringent conditions to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

In another embodiment, the present invention is directed to antisense nucleotides of any of the above-described nucleic acid molecules, in particular, antisense nucleic acid molecules of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13. Preferred antisense molecules include SEQ ID NO: 15 and SEQ ID NO: 17, for example.

In still another embodiment, the present invention provides expression vectors in which any of the foregoing nucleic acid molecules or a fragment thereof has been inserted.

In another embodiment, host cells which are transformed with such an expression vector are provided by the present invention.

In still another embodiment, the present invention provides methods of producing recombinant OGFr proteins or peptide fragments thereof by using the nucleic acid molecules of the present invention.

In a further aspect, the present invention provides isolated proteins, the sequences of which are set forth in SEQ ID NOs: 2, 6, 8, 10, 12 and 14. The present invention also contemplates isolated proteins substantially homologous to any of SEQ ID NOs: 2, 6, 8, 10, 12 and 14.

Also embraced by the present invention are functional equivalents or derivatives of any of SEQ ID NO: 2, 6, 8, 10, 12 and 14.

Another embodiment of the present invention is directed to antibodies raised against an OGFr consisting of any one sequence of SEQ ID NOs: 2, 6, 8, 10, 12 and 14, in particular, monoclonal antibodies.

In a further aspect of the present invention, pharmaceutical compositions are provided in which one or more of the isolated nucleic acid molecules, antisense molecules, expression vectors, cells, isolated OGFr proteins or functional derivatives and antibodies directed against OGFr proteins of the present invention, are included.

In one embodiment, the present invention provides a method of detecting the expression of an OGF receptor in a tissue by using a nucleic acid sequence encoding such OGFr or a portion thereof.

In another embodiment, the present invention provides methods for detecting the level of an OGFr in a tissue by using antibodies, particularly, monoclonal antibodies, that specifically recognize the OGFr.

In one embodiment, the present invention provides methods of inhibiting growth of cells *in vitro* by administering to such cells, an effective amount of nucleic acid molecules coding for an OGFr or a functional derivative thereof.

In another embodiment, the present invention provides methods of promoting growth of cells *in vitro* by administering to such cells, an effective amount of an OGFr antisense molecule.

In another embodiment, the present invention provides methods of promoting growth of cells *in vitro* by administering to such cells, an effective amount of an

antibody directed against an OGFr thereby interfering or inhibiting the function of the OGFr.

In still another embodiment, the present invention provides methods of treating cancers in a patient by enhancing the function of the OGF ligand-receptor system in the cancerous cells.

Cancers which can be treated by the methods of the present invention include, but are not limited to, cancers of neural tissues such as neuroblastoma, prostate cancer, breast cancer, head and neck cancers, gastrointestinal cancers such as pharyngeal, esophageal, stomach, small and large intestine, liver, rectal, colon and pancreatic, biliary tract cancers including gall bladder and bile duct cancers.

In a preferred embodiment, the present invention provides methods of treating cancers in a patient by administering to such patient, an effective amount of a nucleic acid molecule coding for an OGFr or a functional derivative thereof. More preferably, such methods of the present invention are used to treat cancers which are characterized by a deficiency of OGF receptors on the cancerous cells, for example, pancreatic cancer. Desired nucleic acid molecules can be administered in conjunction with OGF.

In a further embodiment, the present invention provides methods of promoting growth of cells in a subject in need thereof by interfering with the function of the OGF ligand-receptor system.

In a preferred embodiment, the present invention provides methods of promoting growth of cells in a subject in need thereof by administering to such subject, an effective amount of an OGFr antisense

molecule or an antibody against OGFr. Such methods can be used in the treatment of wounded tissues, for example.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 depicts tissue distribution of mRNA detected by the cDNA clone of OGFr. Total RNA was isolated from 6-day cerebellum and brain and from adult cerebellum (25 mg/lane) and separated on a 1.2% agarose gel containing 6% formaldehyde and transferred to a nylon membrane and hybridized with [^{32}P]-dCTP labeled cDNA clone #14. A single 2.1 kb mRNA was detected in all tissues examined. The Northern was stripped and hybridized with [^{32}P]-dCTP labeled G3PDH, a constitutively expressed mRNA, to demonstrate equal loading of the RNA samples.

Figure 2 depicts the nucleotide and amino acid sequences of rat OGFr. 5'- and 3'-untranslated regions are included. Repeats are denoted by single and double underlining.

Figure 3 depicts representative saturation isotherm of specific binding of [^{3}H]-[Met^{5}]-enkephalin (square) to purified fusion protein translated *in vitro* from the rat OGFr cDNA clone #12. Mean ± SE binding affinity (K_d) for 6 assays was 2.8 ± 1.1 nM and binding capacity (B_{max}) was 10,530 ± 2,237 fmol/mg protein. Binding was significantly reduced with the addition of 1 mM concentrations of the opioid antagonist naltrexone (circle).

Figure 4 depicts the detection of six-day old (lane 1) and adult (lane 2) rat cerebellar nuclear proteins, native GST protein (lane 3), and recombinant GST-14e protein (lane 4) separated by SDS-PAGE and electrotransferred to nitrocellulose. A: Coomassie blue

stained gel of the electrophoresed proteins. B-D: Western blots stained with polyclonal antibody generated against a 17-kD OGF binding protein (B), antibody made to fusion protein 14e (C), or antibody to GST (D). The staining patterns in panels B and C are similar. The blot in panel D demonstrates the specificity of the fusion protein antibody. The control antibody to GST detected GST and GST-fusion proteins, but not native nuclear homogenate. Arrows indicate the 62, 32, 30 and 17/16 kD OGF binding proteins.

Figure 5 depicts distribution of OGFr in external germinal cells in adjacent midsagittal sections of 6-day old rat cerebellum (A, B) as detected by an antibody to the fusion protein 14e (A) or an antibody to the native 17-kD OGF binding protein (B). Internal granule cells in adult rat cerebellar sections stained with antibody to the fusion protein (14e) (C) revealed no immunoreactivity. Arrows = immunoreactivity. Bar = 50 mm.

Figure 6 depicts histogram of cell number in cultures of IEC-6 rat intestinal epithelial cells treated for 48 hr with either sterile water (CO), 10^{-6} M NTX, 10^{-6} M 23-mer S-ODN (antisense), or scrambled oligoprobe (scrambled). Cells (5 x 10^3) were plated, and compounds and fresh media were added 24 hr and 48 hr later. After 72 hr in culture, cells were trypsinized, stained with trypan blue, and counted with a hemacytometer. Data of different sets are significantly different ($p<0.001$).

Figure 7 depicts representative saturation isotherm and Scatchard plot (inset) of specific binding of [^{3}H]-[Met^5]-enkephalin to a nuclear-enriched fraction

of human placenta. A one-site model of binding was noted.

Figure 8A depicts the nucleotide sequence (SEQ ID NO: 5) and the predicted amino acid sequence (SEQ ID NO: 6) of human OGFr, clone 8; 5'- and 3'-untranslated regions are included.

Figure 8B-8D depicts alternatively spliced forms of OGFr. Colors indicate regions of identity between splice variants.

8B. Nucleotide splicing.

8C. Peptide structure of clones 1 and 127 compared to clone 8. Clone 1 and clone 127 lack the imperfect repeats.

8D. Comparison of repeats in clones #4, 7, and 8. Differences in amino acids are noted in red (presumably due to polymorphisms in the population), and repeats are designated by alternate underlining. Repeats are numbered 1-5, and arrows indicate positions of apparent alternative splicing.

Figure 8E depicts FISH preparation and a companion ideogram (from the International System for Human Cytogenetic Nomenclature, 1995) showing the localization of OGFr to chromosome 20q13.3 (arrow).

Figure 9 depicts Northern blot analysis of the receptor for OGF in human fetal (A) and adult (B) tissues, and cancer cells and tissues (C, D); corresponding β-actin level is shown below each blot.

Figure 10 depicts histogram of cell number in cultures of SK-N-AS human neuroblastoma cells treated for 48 hr with either sterile water (CO), 10^{-6} M NTX, 10^{-6} M S-ODN, or scrambled oligonucleotide. Cells (6×10^3) were

plated and compounds and fresh media added 24 hr and 48 hr later. After 72 hr in culture, cells were trypsinized, stained with trypan blue, and counted with a hemacytometer. Significantly different from CO at $p<0.01$ (***).

Figure 11 depicts the comparison of amino acid similarity between human and rat OGFr. The amino acid similarity is not consistent throughout the OGFr, being higher at the N-terminus. Numbers below boxes indicate amino acid position of the boundaries determined by inspection.

Figure 12 depicts the dependence of binding of [^{3}H-[Met^5]-enkephalin on the protein concentration of PANC-1 nuclear homogenates. Increasing concentrations of protein were incubated with 2 nM radiolabeled [Met^5]-enkephalin in the presence or absence of 100 nM unlabeled [Met^5]-enkephalin for 60 min at 4°C at pH 7.4. Values are means ± SE for at least 2 experiments performed in duplicate.

Figure 13 depicts the dependence of binding of [Met^5]-enkephalin to PANC-1 nuclear homogenates on time and temperature of incubation. Nuclear protein homogenates were incubated with 2 nM [^{3}H]-[Met^5]-enkephalin in the presence or absence of unlabeled [Met^5]-enkephalin (for nonspecific binding) at 4°C, 22°C, or 37°C for varying periods of time. Data are means ± SE for at least 3 experiments performed in duplicate.

Figure 14 depicts the dependence of binding of [^{3}H]-[Met^5]-enkephalin to PANC-1 nuclear homogenates on pH of the incubation buffer. Nuclear protein homogenates were incubated with 2 nM [^{3}H]-[Met^5]-enkephalin in the presence or absence of unlabeled [Met^5]-enkephalin (for

nonspecific binding) at 22°C at a variety of pH levels for the buffer. A pH value of 7.4 appeared to be optimal.

Figure 15 depicts the effects of cations (Na^+, Ca^{++}, Mg^{++}) and guanylylimidodiphosphate (GppNHp) on binding of [^{3}H]-[Met^5]-enkephalin to PANC-1 nuclear homogenates. Histograms represent percentage of maximal binding (mean ± SE) obtained using optimal conditions. Significantly different from optimal binding levels at $p<0.05$ (*) or $p<0.01$ (**).

Figure 16 depicts representative saturation isotherm of specific binding of [^{3}H]-[Met^5]-enkephalin to homogenates of PANC-1 nuclear protein. Mean ± SE binding affinity (K_d) and maximal binding capacity (B_{max}) values from 15 assays performed in duplicate. Representative Scatchard plot (inset) of specific binding of radiolabeled [Met^5]-enkephalin to PANC-1 protein revealed a one-site model of binding.

DETAILED DESCRIPTION OF THE INVENTION

One embodiment of the present invention is directed to isolated nucleic acid molecules. The nucleic acid molecules of the present invention can be of any mammalian origin, including human, pig, dog, monkey, chicken, cow, horse, sheep, murine, rat and the like.

In particular, the present invention provides isolated nucleic acid molecules coding for OGF receptors.

As used herein, an OGF receptor (OGFr) refers to a protein to which the prototypic ligand OGF binds, and through which the growth-related effects of OGF are mediated. OGF is a tonically suppressive autocrine peptide, and its action on growth as an inhibitory agent

is dose-related, reversible, prolonged, independent of serum, and not cytotoxic. The growth-related effects of OGF are associated with cell proliferation, differentiation, and migration, as well as tissue organization. These effects of OGF occur in developing, regeneration, renewing (homeostatsis), wound healing and angiogenesis.

In a preferred embodiment, the present invention provides isolated nucleic acid molecules having SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

A cDNA clone having SEQ ID NO: 1 has been isolated from a λgt11 expression cDNA library constructed from fetal rat brain mRNA. Such cDNA encodes a protein of 580 amino acid (SEQ ID NO: 2).

cDNA clones of SEQ ID NOs: 4-5, 7, 9, 11 and 13 encoding multiple alternatively spliced forms of a human OGF receptor have been obtained by assembling the sequences of 5' and 3' RACE products using human placenta mRNA. The longest assembled clone (SEQ ID NO: 5) encodes a protein of 697 amino acids (SEQ ID NO: 6). SEQ ID NOs: 4-5, 7, 9, 11 and 13 share a portion of the nucleotide sequence at the 5' region, but differ in the 3' region. The polypeptide sequences encoded by these cDNA clones are set forth in SEQ ID NOs: 6, 8, 10, 12 and 14. SEQ ID NO: 5 differs from SEQ ID NO: 4 by having additional 118 nucleotides at the 3' untranslated region.

The present invention also contemplates degenerate sequences of SEQ ID NOs: 1, 4, 5, 7, 9, 11 and 13, i.e., nucleic acid molecules encoding any of the polypeptides of SEQ ID NOs: 2, 6, 8, 10, 12 and 14, which employ alternative codons to those present in SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

Fragments of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 are also provided by the present invention, for example, SEQ ID NO: 3 (a partial rat cDNA clone).

As used herein, "a fragment of a nucleic acid molecule" should be at least about 12, preferably about 15 bp in length. Fragments of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 can be used as probes to screen cDNA libraries for OGFr genes from other species using, e.g., Southern Blot or PCR. Fragments of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 can also be inserted in expression vectors to make the encoded peptides.

In another embodiment, the present invention is directed to nucleic acid molecules that are substantially homologous to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

The phrase "substantially homologous" when referring to nucleotide sequences, denotes the degree of homology of at least 45%, more preferably, at least about 60%, even more preferably, at least about 75%. The degree of homology as used herein is calculated by using the GAP program with a unary comparison matrix, a 3.0 gap penalty, an additional 0.10 penalty for each symbol in each gap, and no penalty for end gaps.

Nucleic acid molecules substantially homologous to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 can be obtained by a variety of well-known techniques. For example, oligonucleotides or DNA fragments can be made from SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13 and employed to screen cDNA libraries for homologous nucleic acid molecules using techniques such as PCR or DNA hybridizations under stringent conditions.

"Stringent conditions" as used herein refer to conditions such as, 18 hours of hybridization at 65°,

followed by four one-hour washes with 2x SSC, 0.1% SDS, and a final wash with 0.2x SSC, more preferably 0.1x SSC, and 0.1% SDS for 30 minutes, as well as alternate conditions which afford the same level of stringency, and more stringent conditions.

Thus, isolated nucleic acid molecules, the complement sequences of which hybridize under stringent conditions to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13, are also included in the present invention.

Nucleic acid molecules that are substantially homologous to the subject nucleic acid molecules or hybridize to the complement sequences of the subject nucleic acid molecules, also include variants of the subject nucleic acid molecules, such as alternative spliced forms and degenerate forms.

Given the nucleic acid molecules encoding OGF receptors, those skilled in the art can readily make modifications, including substitutions, deletions or additions of one or more base pairs, to obtain nucleic acid molecules coding for modified forms of opioid receptors. For example, those skilled in the art can identify the domain(s) that are responsible for the binding of OGF and the domain(s) that interacts with downstream signaling molecules, and thus can make modified forms of OGF receptors that either have superior OGF binding capacities, or can bind OGF but can not interact with downstream signaling molecules so as to accomplish the desired OGF-mediated biological function. Modified OGF receptors can be in the form of substitutions, deletions (including truncations) or insertions of one or more amino acids. Depending on the circumstance, modified forms of OGF receptors can be used

to modulate cell growth, either to stimulate or to inhibit cell growth.

In another embodiment, the present invention is directed to antisense nucleotides of any of the above-described nucleic acid molecules, in particular, antisense nucleic acid molecules of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

In a preferred embodiment, the present invention provides antisense molecules SEQ ID NO: 15 and and SEQ ID NO: 17.

As used herein, the term "antisense nucleotide", "antisense oligonucleotide" or "antisense molecule" refers to an oligonucleotide that hybridizes under physiological conditions to a particular gene or to an mRNA transcript of such gene and, thereby, inhibits the transcription of such gene and/or the translation of such mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene or its mRNA.

It is recognized by those skilled in the art that the exact length of the antisense oligonucleotide and its degree of complementarity with its target depend upon the specific target selected. Preferably, an antisense oligonucleotide is constructed so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions.

Based upon SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13, those skilled in the art can generate appropriate antisense molecules for use in accordance with the present invention. In general, such antisense

oligonucleotides should be at least 7, and preferably, at least about 15 consecutive bases which are complementary to the target DNA or mRNA. Most preferably, the antisense oligonucleotides contain a complementary sequence of 20-30 bases. Although oligonucleotides may be designed according to any region of a gene or its mRNA transcript, preferably, the antisense oligonucleotides are complementary to the 5' region or upstream sites such as translation initiation, transcription initiation or promoter sites.

The antisense oligonucleotides of the invention can be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof, which are covalently linked, as in natural systems, via a phosphodiester internucleoside linkage.

The antisense oligonucleotides of the invention also may include "modified" oligonucleotides. The term "modified oligonucleotide" as used herein refers to an oligonucleotide in which a synthetic linkage other than a natural 5'-3' phosphodiester linkage is present, and/or a modified base or chemical group is present. Synthetic internucleoside linkages include phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, peptides, and carboxymethyl esters. Modified oligonucleotides can include a 2'-O-alkylated ribose group, sugars such as arabinose instead of ribose, base analogs such as C-5 propyne modified bases (Wagner et al., *Nature Biotechnology* 14:840-844, 1996).

In another embodiment, the present invention provides expression vectors in which any of the foregoing

nucleic acid molecules or a fragment thereof has been inserted for purposes of propagation of such nucleic acid molecule and/or production of the polypeptide encoded thereby.

For propagation of a desired nucleic acid molecule, the nucleic acid molecule can be inserted into a vector having a replication origin operable in a desired host, and preferably, a selectable marker, e.g., a marker conferring resistance to an antibiotic. Many of these vectors are available to those skilled in the art, such as pBR322 (New England Biolab), pBluescript (Strategene) and the like. For purpose of expression, a desired nucleic acid molecule can be placed in an operable linkage to a promoter and inserted into a vector appropriate for directing expression in a desired host. For expression in a eukaryotic cell, viral vectors are preferred, e.g., a retroviral, adenoviral, herpes simplex viral vectors or yeast vectors. For expression in a prokaryotic cell, phage vectors are preferred. For expression in an insect cell, baculovirus-based vectors can be used.

In still another embodiment, host cells which are transformed with such an expression vector are provided by the present invention. Those skilled in the art are equally familiar with the choice of cell lines and the procedures to transform such cell lines. Examples of the cell lines include, but are not limited to, eukaryotic cells, e.g., COS cells such as COS-7, CHO cells such as CHO-1, NIH 3T3 cells, yeast cells such as strains of *Saccharomyces* and *Pichia pastoris*, insect cells such as *Spodoptera frugiperda*; and prokaryotic

cells, e.g., strains of *E. coli*, strains of *Pseudomonas* such as *Pseudomonas aeruginosa* or strains of *Bacillus*.

In another embodiment, the present invention provides methods of producing recombinant OGFr proteins or peptide fragments thereof by using the nucleic acid molecules of the present invention.

In accordance with such methods, a nucleic acid molecule encoding an OGFr or a peptide fragment thereof is inserted into an expression vector, which is then transformed into a desired host cell. Choices of expression vectors and host cells have been described herein above. Recombinantly expressed proteins can be purified from the transformed cells following routine procedures.

In a further aspect, the present invention provides isolated proteins, the sequences of which are set forth in SEQ ID NOs: 2, 6, 8, 10, 12 and 14.

According to the present invention, SEQ ID NO: 2 sets forth the amino acid composition of rat OGFr, SEQ ID NO: 6, 8, 10, 12 and 14 set forth the amino acid compositions of 5 alternatively spliced human OGFr, with SEQ ID NO: 6 constituting the longest polypeptide (697 aa).

The present invention also contemplates isolated proteins substantially homologous to any of SEQ ID NOs: 2, 6, 8, 10, 12 and 14. Such homologous proteins can be of any mammalian origin which includes human, pig, dog, monkey, chicken, cow, horse, sheep, murine, rat and the like.

By "substantial homologous" is meant the degree of amino acid similarity of at least about 45%, preferably at least about 60%, and more preferably at

least about 75%. As used herein, the degree of similarity is calculated using the BESTFIT Program (Wisconsin GCG release 8) with the following set of parameters: Gap Weight = 5.0, Length Weight = 0.3, Average Match = 1.0, and Average Mismatch = 0.9.

Also embraced in the present invention are functional equivalents or derivatives of any of SEQ ID NO: 2, 6, 8, 10, 12 and 14.

For purpose of the present invention, "a functional derivative of a protein" includes any modified form of such protein which retains one or more of the biological activities of such protein. A typical biological activity of an OGFr is its specific binding to Met^5-enkephalin, which binding can be reversibly blocked by naltrexone or naloxone. Other biological activities of an OGFr manifest as, e..g, inhibition of cell growth.

The modification of an OGFr can include amino acid deletions, insertions, substitutions or truncations. It is appreciated by those skilled in the art that regions of OGFrs that are well conserved among species may be critical in preserving the biological activities of the OGFrs. Notably in this regard, a prominent feature shared by the isolated proteins of the present invention is the presence of multiple copies of imperfect repeats, as indicated in **Figure 2** and **Figure 8**. In addition, when comparing SEQ ID NO: 2 (rat OGFr) with SEQ ID NO: 6 (human OGFr) (**Figure 11**), a striking similarity is observed in the first 297 amino acids, with 87% being similar and 79% identical. Beyond this point, the number of both similar and identical amino acids drops notably. Thus, a 56% similarity and a 40% identical amino acid profile could be found from amino acids 297 to 464; a

similarity ranging from 43 to 47% , and identical amino acids ranging from 20 to 23% were found thereafter. Therefore, rat OGFr and human OGFr have a great similarity at the N terminus, but dissimilarities at the C terminus.

Another embodiment of the present invention is directed to antibodies raised against an OGFr consisting of any one sequence of SEQ ID NOs: 2, 6, 8, 10, 12 and 14.

The antibodies of the present invention can be generated by using a full-length OGFr protein or a portion thereof as an immunogen. For the purpose of raising antibodies, "a portion of an OGFr protein" refers to a peptide of at least 8 or 9 amino acids. Preferably, the protein or a portion thereof for use as an antigen, is obtained from a recombinant expression system, or chemical synthesis in a standard peptide synthesizer.

Antibodies can be generated by injecting an effective amount of an OGFr protein or a portion thereof into a suitable animal, alone or in combination with an adjuvant. Such animal can include rabbit, chicken, rat, mouse, goat, horse and the like. Both polyclonal antibodies and monoclonal antibodies are contemplated by the present invention. The procedures for making polyclonal and monoclonal antibodies are well known in the art and can be found in, e.g., Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, 1988.

In a further aspect of the present invention, pharmaceutical compositions are provided in which one or more of the isolated nucleic acid molecules, antisense molecules, expression vectors, cells, isolated OGFr

proteins or functional derivatives and antibodies directed against OGFr proteins of the present invention, are included.

The pharmaceutical compositions of the present invention can also include a pharmaceutically-acceptable carrier. As used herein, "a pharmaceutically-acceptable carrier" includes any and all solvents, dispersion media, isotonic agents and the like. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of the active ingredients contained therein, its use in practicing the methods of the present invention is appropriate. The carrier can be liquid, semi-solid, e.g. pastes, or solid carriers. Examples of carriers include oils, water, saline solutions, alcohol, sugar, gel, lipids, liposomes, resins, porous matrices, binders, fillers, coatings, preservatives and the like, or combinations thereof. The carrier for use in the present methods can also be a controlled release matrix, a material which allows the slow release of substances mixed or admixed therein. Examples of such controlled release matrix material include, but are not limited to, sustained release biodegradable formulations described in U.S. Patent 4,849,141 to Fujioka et al., U.S.Patent 4,774,091 to Yamashira, U.S.Patent 4,703,108 to Silver et al., and Brem et al.(*J. Neurosurg.* 74: 441-446, 1991), all of which are incorporated herein by reference.

The pharmaceutical compositions of the present invention can also include other appropriate active ingredients, such as pentapeptide Met^5-enkephalin.

In accordance with the present invention, the active ingredients of the present pharmaceutical

compositions can be combined with the carrier in any convenient and practical manner, e.g., by admixture, solution, suspension, emulsification, encapsulation, absorption and the like, and can be made in formulations such as tablets, capsules, powder, syrup, suspensions that are suitable for injections, implantations, inhalations, ingestions or the like. When appropriate, the pharmaceutical compositions of the present invention should be made sterile by well known procedures. For example, solutions can be made sterile by filter sterilization or autoclave. To obtain a sterile powder, sterilized solutions can be vacuum-dried or freeze-dried as necessary.

In a further aspect of the invention, the nucleic acid molecules encoding an OGFr or a portion thereof, as well as the antibodies against an OGFr, are employed to detect the level or the expression of an OGFr in a tissue or organ. Comparison of the level or the expression of an OGFr within healthy and unhealthy tissues, permit detection of an abnormality of the level or the expression of such OGFr.

In one embodiment, the present invention provides a method of detecting the expression of an OGF receptor in a tissue of a subject by using a nucleic acid sequence encoding the OGFr or a portion thereof.

In general, total RNA can be isolated from a tissue sample of the subject. The level of the OGFr mRNA can be analyzed in various assays, such as Northern Blot Analysis or reverse transcriptase-coupled PCR analysis. The nucleotide sequence for use in these assays should be at least about 15 or 16 base pairs in length. The nucleotide reagent of the present invention can be

detectably labeled, for example, with a radioisotope, a fluorescent compound, or a chemiluminescent compound. The teachings for any of the above-described procedures are well-known to those skilled in the art and can be found in, e.g., *Current Protocols in Molecular Cloning* (Ausubel et al., eds., John Wiley & Sons, New York).

In another embodiment, the present invention provides methods for detecting the level of an OGFr in a tissue by using antibodies, particularly, monoclonal antibodies, that specifically recognize the OGFr.

According to the present invention, the level of an OGFr in a tissue can be determined using an antibody and a variety of *in vitro* assays. Generally, a tissue sample can be taken from the subject, and depending on the assay used, the sample may need to be pretreated. For example, cells can be homogenized, proteins can be extracted from the homogenized cells.

The *in vitro* assays which can be employed herein include, e.g., immunoassays that are based on antigen capture, antibody capture (e.g., ELISA, Western Blot, etc), or two antibody sandwich assay (either forward or reverse mode). Multiple teachings are available for those skilled in the art. See, e.g., Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, 1988.

The antibodies or the proteins isolated from the sample can be utilized in liquid phase or bound to a solid phase carrier. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified cellulose, polyacrylamides, agaroses and magnetite. In addition, the antibodies or the proteins isolated from the tissue

sample can be detectably labeled in various ways for a quantitative determination. Examples of detectable labels include secondary antibodies, enzymes such as horseradish peroxidase, β-galactosidase or alkaline phosphatase, fluorophores or radioisotope.

In accordance with the present invention, antibodies can also be used *in vivo* for detecting the level of an OGFr in a subject. Monoclonal antibodies are preferred for *in vivo* detection.

In using a monoclonal antibody for *in vivo* detection, the monoclonal antibody is detectably labeled, e.g., with a radioisotope. The skilled artisan can chose a radioisotope according to the type of detection instrument that is available. The chosen radioisotope should have a type of decay which is detectable for a given type of instrument. Preferably, the chosen radioisotope has a half life that is long enough for detection at the time of maximum uptake by the subject, but short enough to minimize deleterious radiation to the subject. A radioisotope suitable for *in vivo* imaging methods of the present invention does not have a particle emission, but produces a large number of photons in the 140-250 keV range which may be readily detected by conventional gamma cameras.

According to the present invention, radioisotopes may be coupled to immunoglobulin by using an intermediate functional group, particularly for binding metallic ion-type of radioisotopes to immunoglobulins. Appropriate intermediate functional groups include the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar

molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

A monoclonal antibody suitable for use in the present methods can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

In accordance with the present invention, a labeled monoclonal antibody can be administered in any appropriate manner, such as via an oral, ophthalmic, nasal, transdermal, parenteral (e.g., intravenous, intraperitoneal, intradermal, subcutaneous or intramuscular), intratumor, intraembryonic, or intrafetal route, to the subject of interest in a quantity that is sufficient to enable a specific detection of the OGFr, and that allows rapidly clearance of the reagent from the subject in order to give the best target-to-background signal ratio. The dosage of detectably labeled monoclonal antibody can vary depending on such factors as age, gender, and severity of the disorder of the subject, or the subject's response to a therapeutic regimen. Those skilled in the art can determine the appropriate dose of a monoclonal antibody reagent using conventional techniques. As a general rule, the dosage of a monoclonal antibody can fall in the range of about 0.001 mg/m^2 to about 500 mg/m^2, preferably 0.1 mg/m^2 to about

200 mg/m^2, most preferably about 0.1 mg/m^2 to about 10 mg/m^2. *In vivo* imaging techniques are described in U.S. Patent No. 4,036,945 and No. 4,311,688, the disclosures of which are incorporated herein by reference.

In a further aspect of the present invention, the nucleic acid molecules encoding an OGFr or functional derivatives thereof or modified forms thereof, the antisense molecules and antibodies directed against an OGF, are employed to modulate cell growth.

An OGF receptor protein acts together with its native ligand, opioid peptide [Met^5]-enkephalin (or OGF), to inhibit cell growth. According to the present invention, such ligand-receptor signaling system can be manipulated to achieve desired effects under various physiological circumstances. For example, in cases of cancer, it is desirable to enhance or potentiate the activity of the OGF ligand-receptor signaling system in cancerous cells thereby inhibiting the growth of the cancerous cells. On the other hand, in cases of cellular renewal or regeneration, wound healing or angiogenesis, for example, it maybe desirable to inhibit or reduce the activity of the OGF ligand-receptor signaling system in cells of the desired tissues, thereby enhancing or accelerating the growth of such cells. Thus, the term "modulating" as used herein refers to regulating and manipulating to enhance or reduce the activity of the OGF ligand-receptor signaling system.

In one embodiment, the present invention provides methods of inhibiting growth of cells *in vitro* in need of such inhibition by introducing to such cells *in vitro*, an effective amount of nucleic acid molecules coding for an OGFr or a functional derivative thereof.

The nucleic acid molecules can be introduced to such cells by well-known procedures, e.g., transfection. Such nucleic acid molecules can be introduced to the cells in conjunction with Met^5-enkephalin.

In another embodiment, the present invention provides methods of promoting growth of cells *in vitro* in need of such promotion by introducing to such cells, an effective amount of an OGFr antisense molecule. The antisense molecules can be introduced to the cells by, e.g., simply adding such antisense molecules to the culture media of the cells.

In another embodiment, the present invention provides methods of promoting growth of cells *in vitro* in need of such promotion by introducing to such cells, an effective amount of an antibody directed against an OGFr thereby interfering or inhibiting the function of the OGFr. The antibodies can be introduced to the cells by, e.g., simply adding such antibody to the culture media of the cells.

In still another embodiment, the present invention provides methods of treating cancers in a patient by enhancing the function of the OGF ligand-receptor system in the cancerous cells.

In a preferred embodiment, the function of the OGF ligand-receptor system in the cancerous cells can be enhanced by providing the cancerous cells additional nucleic acid molecules encoding OGF receptors.

Thus, the present invention provides methods of treating cancers in a patient by administering to such patient, an effective amount of nucleic acid molecules coding for an OGFr or a functional derivative thereof.

For the purposes of the present invention, the term "treating" means preventing the onset of cancer, inhibiting the growth of existing cancer, preventing the recurrence of cancer, or arresting cancer completely.

Cancers which can be treated by the methods of the present invention include, but are not limited to, cancers of neural tissues such as neuroblastoma, prostate cancer, breast cancer, head and neck cancers, gastrointestinal cancers such as pharyngeal, esophageal, stomach, small and large intestine, liver, rectal, colon and pancreatic, biliary tract cancers including gall bladder and bile duct cancers.

More preferably, the methods of the present invention are used to treat cancers which are characterized by a deficiency of OGF receptors on the cancerous cells. According to the present invention, an example of such cancer is pancreatic cancer.

A deficiency of OGF receptors can be due to insufficient expression of the receptors or expression of non-functional receptors, as compared with controls, e.g., adjacent normal cells from the same individual or cells from pooled normal individuals.

A variety of assays can be used to determine whether there is a deficiency of OGF receptors in subject cells. Such assays include those described herein above for detecting the level or the expression of OGFr receptors, e.g., Northern Blot or RT-PCR using nucleic acid molecules, and immunoassays using antibodies. Other assays include binding assays which determine the Met^5-enkephalin binding capacity of subject cells or protein extracted therefrom. Examples of such binding assays are provided hereinafter (Example 6).

Preferably, a nucleotide sequence encoding an OGF or a functional derivative thereof for use in treating cancers is provided in an expression vector, e.g., a typical gene therapy vector. Preferred gene therapy vectors include retroviral, adenoviral, herpes simplex viral, adeno-associated viral and vaccinia vectors. Examples of retroviral vectors include, but are not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV)-derived recombinant vectors. More preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), thereby providing a broader host range than murine vectors, for example. Gene therapy vectors can be made tissue specific by, for example, linking the nucleotide sequence to a tissue-specific promoter. Multiple teachings of gene therapy are available for those skilled in the art, e.g., W.F. Anderson (1984) "Prospects for Human Gene Therapy" *Science 226*: 401-409; S.H. Hughes (1988) "Introduction" *Current Communications in Molecular Biology 71*: 1-12; N. Muzyczka and S. McLaughlin (1988) "Use of Adeno-associated Virus as a Mammalian Transduction Vector" *Communications in Molecular Biology 70*: 39-44; T. Friedman (1989) "Progress Toward Human Gene Therapy" *Science 244*: 1275-1281 and W.F. Anderson (1992) "Human Gene Therapy" *Science 256*: 608-613.

The amount of a nucleic acid molecule to be therapeutically effective can be determined according to the age and the condition of the subject.

Desired nucleic acid molecules can be administered in conjunction with OGF. Other appropriate

materials, such as pharmaceutical carriers, can be administered together with the nucleic acid molecules as well. Any of the foregoing described pharmaceutically acceptable carriers can be used, and can be admixed with the nucleic acid molecules in the manner described hereinabove.

The administration of the desired nucleic acid molecules, either alone or with other appropriate material, may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. Preferably, the administration is carried out by injection, including subcutaneous, intradermal, intramuscular, transdermal, intraperitoneal (i.p.), intra-arterial (i.a.), intravenous (i.v.) injection, or direct injection into the tumor. Multiple administrations may be required, which can be determined by a physician.

In a further embodiment, the present invention provides methods of promoting growth of cells in a subject in need thereof by interfering with the function of the OGF ligand-receptor system.

According to the present invention, the function of the OGF ligand-receptor system can be inhibited by using antisense molecules which interfere with the expression of OGF receptors, or by using antibodies against OGF receptors which interfere, via steric hindrance, e.g., with the function of OGF receptors.

Accordingly, In a preferred embodiment, the present invention provides methods of promoting growth of cells in a subject in need thereof by administering to

such subject, an effective amount of an OGFr antisense molecule or an antibody against OGFr.

Such methods of the present invention can be used to assist in the healing of wounded tissues or organs, including but not limited to the skin, the cornea, liver, uterus, nerves, subcutaneous tissues, mucosal tissues, intestinal tissues, and fetal tissues.

Antisense molecules for use in the methods of the present invention can be placed on expression vectors, such as gene therapy vectors described hereinabove.

The amount of an antisense molecule or an antibody to be therapeutically effective can be determined according to the age and the condition of the subject.

Both antisense molecules and antibodies can be administered alone or in combination with one or more pharmaceutically-acceptable carriers. Such carriers have been described hereinabove. Antisense molecules or antibodies can be admixed with one or more carriers in manners described hereinabove, and then administered via any of the foregoing routes.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. The terms and expressions which have been employed in the present disclosure are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof. It is to be understood that various modifications are possible within the scope of the

invention. All the publications mentioned in the present disclosure are incorporated herein by reference.

5

Example 1

Isolation of a cDNA Clone Encoding a Rat OGF Binding Protein

An antibody against the rat OGF binding protein (BO461) was produced according to published procedures (Zagon et al., *Brain Res.* 630: 295-302, 1993). In brief, 2-dimensional gels of the nuclear fraction (P1) from a 6-day old rat cerebellum were transferred to nitrocellulose, and ligand blotting with [^{125}I]-[Met^{5}]-enkephalin was performed to identify an OGF binding protein (Zagon et al., *Brain Res.* 482: 297-305, 1989). The protein was electroeluted and injected into New Zealand white rabbits to generate polyclonal antibodies. Serum was collected and purified using ammonium sulfate precipitation and DEAE Affi-blue gel filtration.

One million plaques from a λgt11 expression cDNA library (oligo-dT primed, original complexity of 1.6 x 10^{6} pfu with insert sizes ranging from 0.6 to 4.0 kb), constructed from 18-day old fetal rat brain mRNA (Clontech, Palo Alto, CA), were screened using the polyclonal antibody that recognized the OGF binding protein. Immunoreactive plaques were identified by reaction with horseradish peroxidase or [^{125}I]-protein A. Thirty-two plaques reacted positively to the BO461 antiserum, of which four were purified and subjected to restriction digestion. One of these plaques, designated clone #14 (SEQ ID NO: 3), was characterized further.

The 1000 bp cDNA insert from clone #14 was labeled with [^{32}P]dCTP and used to probe Northern blots of total RNA isolated from 6-day old and adult rat cerebellum. Northern blot analysis was performed

according to McLaughlin and Allar (*Mol. Brain Res.* 60: 160-167, 1998). In brief, RNA was isolated from 6-day old and adult rat tissues. Membranes were hybridized at 42°C for 16-18 hr in fresh prehybridization buffer containing 10^6 cpm/ml of random prime labeled clone #14 cDNA. Filters were subjected to final washes at 60°C with 0.1 X SSC containing 0.1% SDS for 30 min, wrapped in plastic while wet, and exposed to autoradiography film with intensifying screens for 2-4 days at -70°C. To control for differences in the amount of RNA loaded, as well as the integrity of RNA, blots were stripped and probed with [^{32}P]-labeled cDNA for G3PDH. As shown in **Figure 1**, the cDNA probe detected a species of rat mRNA that was 2.1 kb, expressed abundantly in 6-day old cerebellum and brain, but at low levels (3-fold less than at 6-days) in adult cerebellum.

Since the #14 clone was not full length, labeled #14 cDNA was used as a hybridization probe to screen the λgt11 fetal rat brain library for full-length clones. Thirteen positive clones were identified and purified from the library by colony hybridization. Digestion of the purified clones with *EcoR1* released a full size insert of 2.1 kb from clone #12. The #14 and the #12 cDNAs were sequenced in both directions. **Figure 2** shows the nucleotide sequence (SEQ ID NO: 1), and the deduced amino acid sequence (SEQ ID NO: 2), of the full length cDNA, #12; 5' and 3' untranslated regions have been included. The open reading frame was found to encode a protein of 580 amino acids, with 8 imperfect repeat units of 9 amino acids at positions 467 to 538. The molecular weight as calculated from the sequence is

58 kD. Search of the sequences in GenBank revealed no homology to this cDNA.

Example 2

Characterizing the OGF Receptor

Binding Assays

Inserts from clone #14 encoding the C-terminal 197 amino acids of rat OGFr, and from clone #12 encoding all 580 amino acids of rat OGFr, were ligated into the pGEX-3X expression vector (Promega, Madison, WI) to generate glutathione-S-transferase (GST) fusion proteins, referred to herein as 14-GST and 12-GST, respectively. When expressed in XL1-Blue strain bacteria (Stratagene, La Jolla, CA), and induced with isopropyl b-o-thiogalactopyranoside for 3 hr, these plasmids produced GST-OGF-binding fusion proteins which were then purified from crude bacterial extracts using glutathione affinity chromatography and 1-dimensional PAGE gel electrophoresis.

These fusion proteins were used in binding assays with [^{3}H]-[Met^5]-enkephalin (Zagon et al., *Brain Res.* 551: 28-35, 1991; Zagon et al., *Brain Res.* 605: 50-56, 1993). Fusion proteins (80-120 ng/tube) were incubated for 30 min with shaking at 22^{0}C. Non-specific binding was measured in the presence of unlabeled [Met^5]-enkephalin. In some assays, NTX (10^{-3} M) was added to the reaction mixture to monitor opioid antagonist blockade of binding. Binding was terminated by filtering homogenates through Whatman GF/B filters. Saturation isotherms were plotted using GraphPad Prism software (GraphPad Software Inc., San Diego, CA).

Specific competition of 12-GST fusion protein was determined in the presence of a range (10^{-10} to 10^{-3} M)

of ligands including [D-Ala^2,MePhe^4,Glyol^5]-enkephalin (DAMGO), [D-Pen^{2,5}]-enkephalin (DPDPE), dynorphin A1-8, U69,583, and morphine sulfate (**Figure 3**). Specific and saturable binding was observed, with a mean binding affinity (K_d) of 2.8 ± 1.1 nM and binding capacity (B_{max}) of 10,530 ± 2,237 fmol/mg protein. Addition of naltrexone to the preparations significantly reduced specific and saturable binding, with reductions in B_{max} of 83% noted (**Figure 3**). Representative Scatchard plot of specific binding of radiolabeled [Met^5]-enkephalin revealed a one-site model of binding.

Using a variety of ligands that recognized classical opioid receptors, no competitive binding (>10^{-3} M) for radiolabeled [Met^5]-enkephalin by DAMGO or morphine sulfate (μ receptor), DPDPE (δ receptor), dynorphin A1-8 and U69,583 (κ receptor) was observed.

Western Blotting

Antibodies against the recombinant fusion proteins were generated by inoculating New Zealand white rabbits with 14-GST or with 12-GST fusion proteins suspended in Freund's adjuvant. The GST was not cleaved from the proteins prior to inoculating the rabbits, allowing the GST to function as a carrier protein. Rabbits were injected every 4 weeks for 2 months, tested for their titer, and exsanguinated 4 days after the final injection.

Nuclear preparations of 6-day and adult rat cerebellum, as well as GST and 14-GST fusion proteins, were isolated by 12.5% SDS PAGE, and electrotransferred to nitrocellulose. Western blotting performed with primary antibodies to either the 14-GST fusion protein

(Zagon et al., *Brain Res.* 630: 295-302, 1993), native OGF binding protein, or GST; [^{125}I]-protein A was used for antibody detection.

Antibodies to the recombinant fusion protein derived from clone #14 (14-GST) were titered and 1:1000 dilutions detected 10 ng of fusion protein. When reacted with nuclear preparations of 6-day old cerebellum on a 1-dimensional Western blot, anti-14-GST recognized 5 polypeptides: 62, 32, 30, 17, and 16 kD, as well as the recombinant protein (**Figure 4**). Western blots stained with antibodies generated against the native 32 kD binding protein detected the 62, 32, 30, 17, and 16 kD polypeptides, in addition to the recombinant protein (**Figure 4**). The antibody to the recombinant fusion protein or the native 32 kD polypeptide stained homogenates of the adult rat cerebellum, but was of a notably lesser density than in the 6-day specimen (**Figure 4**).

Immunocytochemistry

Immunocytochemistry was performed using methodology described previously (Zagon et al., *Brain Res.* 803: 61-68, 1998). In brief, rat brains from 6-day old and adult animals were frozen and sectioned. Adjacent sections were stained with antibodies to 14-GST fusion protein or with polyclonal antibodies to the OGF binding protein. Controls included staining sections with primary antibodies preabsorbed with either 14-GST fusion protein or with OGF binding protein, as well as secondary antibodies only.

The staining pattern in immunocytochemical preparations employing antibodies to the recombinant

fusion protein was similar to that observed when using antibodies to the authentic binding protein (**Figure 5**). Both antibodies revealed immunoreactivity in the 6-day old rat cerebellum, with cells of the external germinal layer exhibiting prominent staining of the cytoplasm and low reactivity of the nucleoplasm. The internal granule cells of adult rat cerebellar tissues demonstrated little specific immunoreactivity with either antibody.

Example 3

Regulation of Cell Growth Using Rat OGFr Antisense DNA

In order to study the function of the isolated cDNA with respect to growth, a 23-mer antisense S-ODN (nuclease-resistant phosphorothioate; Oligo, Etc., Bethel, ME) targeted against a sequence containing the translation initiation site of the OGF binding protein was designed: 5'- GACTCAGGGACTTAGCTTCATCC-3' (antisense, SEQ ID NO: 15). A 23-mer with a scrambled sequence was also designed: 5'-ATAGATACTACGCCGGCTGTCCT-3' (scrambled, SEQ ID NO: 16).

The IEC-6 rat small intestine epithelial cell line (American Type Tissue Culture Collection, Manassas, VA) were grown in Dulbecco's medium supplemented with 10% fetal calf serum. For experiments, 5 x 10^3 cells/well in a 24-well plate were seeded and, 24 hr later, 10^{-6} M concentrations of the antisense or the scrambled S-ODN were added. Some wells were exposed to 10^{-6} NTX or an equivalent volume of sterile water. Media, S-ODNs, or drugs were changed daily. After 72 hr in culture, cells were trypsinized, stained with trypan blue, and counted with a hemacytometer; 3 wells/treatment group were assessed. The data were evaluated with ANOVA, and subsequent comparisons were made with the Newman-Keuls tests.

As shown in **Figure 6**, the antisense S-ODN elevated cell number by 294% from control cultures within 48 hr of exposure. The cell cultures treated with the scrambled probe were similar in growth to control levels. Cultures treated with NTX, an antagonist to the opioid

binding protein, also demonstrated increased cell growth from control values; the NTX group had 223% more cells than control cultures. The cells treated with scrambled message were comparable to controls in cell number.

Example 4

Isolation of cDNA Encoding a Human OGF Binding Protein

A fragment of the human OGFr cDNA was obtained by reverse transcriptase - polymerase chain reaction (RT-PCR) using mRNA from a pool of 24 male/female, 20-25-week old human fetal brains obtained from spontaneous abortions (Clontech, Palo Alto, CA). Primers were generated from the rat cDNA sequence (clone #12, Example 1). A RT-PCR product of 400 nucleotides (as predicted) was amplified and designated "H5". Homology with rat OGFr was confirmed by Southern blot analysis using [^{32}P]-dCTP labeled rat clone #12. H5 was cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced. RNA from a variety of human tissues was probed with [^{32}P]-dCTP labeled H5, and revealed that the greatest amount of target mRNA was associated with human placenta.

Binding assays were performed on human placenta using radiolabled [Met^5]-enkephalin to characterize this tissue. Binding studies with human placenta and radiolabeled [Met^5]-enkephalin revealed specific and saturable binding, with a mean binding affinity (K_d) of 12.3 ± 3.9 nM and a binding capacity (B_{max}) of 247 ± 95 fmol/mg protein (**Figure 7**). A one-site model of binding was noted.

Using human placental mRNA (Clontech), the complete sequence of human OGFr was assembled with a combination of 3'- and 5'-RACE techniques performed with a Marathon cDNA Amplification Kit (Clontech). RACE products were cloned utilizing a TOPO TA cloning kit (Invitrogen) and sequenced by an automated sequencer (ABI

Prism Model 377 Version 2.1.1) located in the MacroMolecular Facility of The Pennsylvania State University College of Medicine. Sequence data were analyzed with the Sequence Analysis Software Package of the Genetics Computer Group (University of Wisconsin Biotechnology Center).

5' RACE consistently yielded a single species of cDNA, while the 3' RACE revealed extensive alternative splicing. When assembled, the longest predicted cDNA (designated clone 8 for the particular 3' clone found with a given structure) was 2.4 kb. **Figure 8A** shows the nucleotide (SEQ ID NO: 5) and the deduced amino acid sequence (SEQ ID NO: 6) of this cDNA; 5' and 3' untranslated regions have been included. The open reading frame was found to encode a protein of 697 amino acids, and 8 imperfect repeats of 20 amino acids were a prominent feature. The predicted initiation site was flanked by a strong Kozak consensus sequence. A number of alternate spliced forms were detected by 3' RACE, and these are depicted in **Figure 8B-D.** Two of the alternate spliced forms (clones #1 and 127) were missing the imperfect repeats (**Figure 8C**). Clones #4, 7, and 8 differed only in the number of imperfect repeats (**Figure 8D**).

Sequence similarity was compared to the entries recorded in the GenBank database using FASTA and BLAST databases.

The chromosomal location of the human receptor for OGF was determined by FISH as 20q13.3 (**Figure 8E**).

Example 5

Expression Pattern of Human OGFr

Northern blot analysis of human fetal and adult tissues, as well as cancer tissues and cell lines, was performed with **a radiolabeled OGFr cDNA cocktail** according to the procedure described by McLaughlin and Allar (*Mol. Brain Res.* 60: 160-167, 1998).

Briefly, commercially prepared blots of poly A^+ RNA from human tissues and cells were obtained from Clontech (Palo Alto, CA). RNA samples of fetal tissue (Human Fetal II Multiple Tissue Northern (MTN™) Blot, Lot. #080009) were pooled from tissues ranging from 7 to 32 individuals that were 16 to 32 weeks in age, and representative of both genders. The cause of death was spontaneous abortion. The blot of human tissues (Human Multiple Tissue Northern (MTN™) Blot, Lot #8070732) contained pooled samples from 2 to 18 individuals that were 10 to 69 years in age and, except for the heart (males) and placenta (females), was representative of both genders. In the case of the liver, the tissue was obtained from a 35-year-old male. With the exception of the placenta, the causes of death were reported as sudden death (brain, lung, liver, and skeletal muscle) or trauma (heart, kidney, and pancreas). A Human Cancer Cell Line Multiple Tissue Northern (MTN™) Blot (Lot #9050001) was purchased from Clontech and contained: promyelocytic leukemia, HL-60, HeLa cell S3, chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549, and melanoma G361. Cancer cell lines

were purchased from the American Type Tissue Collection (Manassas, VA) and included: human pancreatic cancer cell lines BxPC3, PANC-1, and MIA PaCa-2, squamous cell carcinoma of the head and neck CAL-27, and neuroblastoma SK-N-SH. Human pancreatic total RNA was purchased from InVitrogen (Lot #7110156). Surgical specimens of human tissues included: squamous cell carcinoma of the tongue (65-yr-old male, Stage III), renal cell carcinoma and normal kidney (73-yr-old male, radical nephrectomy).

Total RNA was prepared by immersing preparations in ice-cold 4 M guanidinium isothiocyanate/0.83% β-mercaptoethanol/0.3 M sodium acetate and homogenizing with a polytron (setting 6, 2 x 10 sec). Homogenized tissues were layered over a cushion of 5.7 M cesium chloride/0.3 M sodium acetate and centrifuged at 105,000 x g with an SW55Ti rotor for 18 hr at 25°C. Total RNA was precipitated with ethanol, and quantitated by UV spectrometry. Inasmuch as possible, equal amounts of total RNA were subjected to electrophoresis on 1.0% agarose-2.5% formaldehyde gels, transferred by capillary blotting to nylon membranes, and baked *in vacuo* at 80°C for 1 hr. Filters were prehybridized for 4-8 hr at 42°C in a solution containing 50% deionized formamide, 5 X Denhardt's buffer (50 X Denhardt's = 5 g Ficoll, 5 g polyvinylpyrrolidone, and 5 g bovine serum albumin in 500 ml sterile water), 50 mM sodium phosphate, pH 6.5, 5 X SSC (20 X SSC = 3 M sodium chloride and 0.3 M sodium citrate), 500 mg/ml salmon sperm DNA (Sigma) and 1% SDS.

Membranes were hybridized at 42°C for 22 hr in fresh prehybridization buffer containing 10^6 cpm/ml of random primed human cDNAs. Filters were subjected to

final washes at 60°C with 0.1 X SSC containing 0.1% SDS for 30 min, and wrapped in plastic while wet, and exposed to autoradiography film with intensifying screens for 2-4 days at -70°C. To control for differences in the amount of RNA loaded, as well as the integrity of RNA, blots were stripped and probed with [^{32}P]-labeled cDNA for β-actin (Clontech).

As shown in **Figure 9**, in the human fetal tissues, transcript sizes of 1.7 and 2.4 kb were observed, whereas in the adult tissues and cancer cell lines and tissues only a 2.4 kb mRNA was detected. Receptor for OGF was of low abundance only in adult lung.

Example 6

Regulation of Cell Growth Using Human OGFr Antisense DNA

To study the function of the isolated cDNA with respect to regulation of cell growth, a 23-mer antisense S-ODN (nuclease-resistant phosphorothioate; Oligo, Etc., Bethel, ME) targeted against a sequence containing the translation initiation site of the OGF binding protein was designed: 5'-GGTCGTCCATGCTCGGCTAGAAT-3' (antisense, SEQ ID NO: 17). A scrambled (control) S-ODN was also designed: 5'-GTGCAGTGCAATGCTCTCCGTGA-3' (SEQ ID NO: 18).

The SK-N-AS human neuroblastoma cell line (American Type Tissue Culture Collection, Manassas, VA) was grown in Dulbecco's medium supplemented with 10% fetal calf serum. For experimentation, 6×10^3 cells/well in a 24-well plate were seeded and, 24 hr later, 10^{-6} M concentrations of the antisense or the scrambled S-ODN was added. Some wells were exposed to 10^{-6} M NTX or an equivalent volume of sterile water. Media, S-ODNs, or drugs were changed daily. After 72 hr in culture, cells were trypsinized, stained with trypan blue, and counted with a hemacytometer. Data from the antisense experiments were evaluated with ANOVA, and subsequent comparisons made with the Newman-Keuls tests.

As shown in **Figure 10**, the antisense S-ODN elevated cell number by 60% from control cultures within 48 hr of exposure. The cell cultures treated with the scrambled probe were similar in growth to control levels. Cultures treated with NTX, an antagonist to the opioid

binding protein, also had increased cell growth from control values.

Example 7

Characterization of OGFr in Human Pancreatic Adenocarcinoma

Materials and Methods

Cell culture. PANC-1, Mia PaCa-2, BxPC-3, and Capan-1 cell lines were obtained from the American Type Culture Collection (Manassas, VA). Specific characteristics of each human pancreatic tumor cell line have been reported previously (Zagon et al., *Int. J. Oncol.* 14: 577-584, 1999). PANC-1 and MIA PaCa-2 cells were maintained in Dulbecco's modified media, while Capan-1 and BxPC-3 cells were grown in RPMI media; all media contained 10% fetal calf serum, 2 mM L-glutamine, 1.2% bicarbonate and antibiotics (5,000 Units/ml penicillin, 5 mg/ml streptomycin, 10 mg/ml neomycin). Cells were grown in a humidified atmosphere of 5% CO_2/95% air at 37°C. Panc-1 cells between passages 62-74 were used for the characterization studies.

Preparation of protein fractions. Cell cultures were harvested by scraping of the flasks, and cells were pelleted by centrifugation. The washed pellet was homogenized (Polytron, setting 5, 2 x 10 sec) in a 1:20 (wt/vol) solution of cold 50 mM Tris-HCl with 0.1 mg/ml bacitracin, 1 mg/ml leupeptin, 6 nM thiorphan, 1 mM EGTA, and 3.5 mM PMSF (pH 7.4) at 4°C; this buffer is termed Tris/all. Homogenates were layered over a 1.4 M sucrose cushion, and centrifuged (2,200 g) for 20 min; this step was conducted twice in order to obtain a nuclear pellet (P1).

For subcellular fractionation studies, the supernatant of P1 was centrifuged at 39,000 x g for 30

min to obtain a plasma membrane pellet (P2). The P2 supernatant was centrifuged overnight (100,000 x g), resulting in a microsomal pellet (P3) and soluble supernatant (S3). All fractions were inspected for purity by phase-contrast microscopy.

Receptor binding assays. Homogenates of nuclear protein were diluted with Tris/all to the appropriate protein concentration and incubated at room temperature (22°C) for 20 min to remove endogenous peptides. Aliquots of protein were resuspended to 0.95 ml and incubated with agitation with radioactive ligand. Saturation assays were conducted with various concentrations of ligand, usually ranging from 0.1 to 15 nM. The final volume of the incubation mixture was 1 ml. Isotope incubation was terminated by rapid filtration through Whatman GF-B filters under vacuum pressure with a Brandel Cell Harvester. Filters were rinsed with ice-cold Tris/all buffer, dried at 60°C for 1 hr and counted by liquid scintillation spectrometry (Beckman LS-2800). Nonspecific binding was determined in the presence of 100 nM of [Met^5]-enkephalin. Duplicate tubes of homogenates were assayed for each concentration utilized. Protein concentrations were determined by the BioRad method with g-globulin as the standard.

For competition studies, nuclear homogenates were incubated with 1 nM radiolabeled [Met^5]-enkephalin and a range of concentrations (10^{-4} to 10^{-10} M) of each of the non-labeled compounds. Each concentration was run in triplicate and every compound was tested twice.

Analysis and statistics. Receptor binding data were analyzed with either the Lundon I (Saturation Isotherm Binding Analysis) computer program (Lundon

Software, Cleveland, OH) or GraphPad Prism software. Both programs utilize nonlinear least-squares regression. Binding isotherms and Scatchard plots were computed directly by the programs. Competition data were analyzed by the Lundon II competition data-analysis program, and the inhibition constant was calculated from the half-maximal displacement (concentration inhibiting 50% of maximal response) values using the method of Cheung and Prusoff (*Biochem. Pharmacol.* 22: 3099-3108, 1973). Comparisons of B_{max} and K_d values were made using analysis of variance and posthoc Newman-Keuls tests.

Characterization of [^{3}H]-[Met^5]-enkephalin binding

The optimal conditions for binding of [^{3}H-[Met^5]-enkephalin (OGF) to PANC-1 nuclear (P1) homogenates were determined. Specific binding of radiolabeled ligand to PANC-1 nuclear homogenates was dependent on protein concentration and was linear between 200 and 550 mg/ml (**Figure 12**). Binding of [^{3}H]-[Met^5]-enkephalin to P1 homogenates of PANC-1 cells was also dependent on time and temperature of incubation (**Figure 13**). Maximal specific binding occurred at 22°C, reaching an equilibrium between 60-75 min. Binding at 0°C and 37°C was 12% and 21%, respectively, of the optimal binding at 22°C. The binding of radiolabeled [Met^5]-enkephalin was also dependent on pH of the buffer solution, with an optimal pH of 7.4 being recorded (**Figure 14**).

The effects of monovalent and divalent cations on [^{3}H]-[Met^5]-enkephalin binding to PANC-1 nuclear homogenates are presented in **Figure 15**. Addition of NaCl, $CaCl_2$, and $MgCl_2$ at concentrations of 50, 100, or 200 mM reduced specific radioactive binding by as much as

85%. The binding of [^{3}H]-[Met^5]-enkephalin was not markedly altered by addition of 50, 100, or 200 μM GppNHp to the binding assays.

By use of the optimal conditions for protein concentration, time, temperature, and pH described above, in a buffer containing a cocktail of protease inhibitors, [^{3}H]-[Met^5]-enkephalin binding to PANC-1 nuclear homogenates (P1 fraction) was found to be specific and saturable (**Figure 16**). Computer analysis of binding showed that the data best fit a one-site binding model with an average equilibrium dissociation constant (K_d) of 1.2 ± 0.3 nM and a mean maximal binding capacity (B_{max}) of 36.4 ± 4.1 fmol/mg protein. Nonspecific binding was calculated to be ~52% of the total binding.

Competition assays

Competition experiments using 1 or 2 nM [^{3}H][Met^5]-enkephalin and a range of natural and synthetic opioid peptides were performed to examine the specificity and relationship of radiolabeled [Met^5]-enkephalin to its binding site (**Table I**). [Met^5]-enkephalin exhibited the greatest potency of any of the compounds tested, having 100-fold greater ability to displace [^{3}H][Met^5]enkephalin than the next ranking compound; the competition value (IC_{50}) for [Met^5]-enkephalin was 5.5 nM. Ligands related to the μ- (i.e., DAMGO), δ-(i.e., DPDPE, ICI-174,468), κ-receptors (i.e., dynorphin A, U69,598, ethylketocyclazocine), and well as morphine and leucine enkephalin exhibited little or no competition.

Table I.

Potency of prototypic opioid ligands to compete for binding of [^{3}H]-[Met^5]- enkephalin in nuclear homogenates of human pancreatic tumor cells (PANC-1).

	Compound	$IC_{50}/_{Kd}$
μ Receptor	DAMGO	$> 10^{-4}$
δ Receptor	DPDPE	$> 10^{-4}$
	ICI-174,568	$> 10^{-4}$
κ Receptor	Dynorphin A1-8	$> 10^{-4}$
	U69,598	$> 10^{-5}$
	EKC	$> 10^{-3}$
Other ligands	Morphine	$> 10^{-3}$
	Leucine enkephalin	$> 10^{-4}$
	β-endorphin	$> 10^{-4}$

Data represent values from at least 2 independent assays. Equilibrium dissociation constant for [Met^5]-enkephalin was 1.2 ± 0.3 nM. IC_{50} is the concentration that inhibits 50% of maximal response; $_{KD}$ is the inhibition constant. DAMGO = [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin, DPDPE = [D-Pen^2, Pen^5]-enkephalin, EKC = ethylketocyclazocine.

Subcellular fractionation studies

Sucrose gradient centrifugation was performed to separate the nuclear (P1), membrane (P2), microsomal (P3), and soluble (S3) fractions (**Table II**). In these experiments, specific and saturable binding of [^{3}H]-[Met^5]-enkephalin was detected in the P1 fraction, and computer analysis revealed a one-site model of binding with a K_d of 2.3 ± 0.5 nM and B_{max} of 21.8 ± 4.8 fmol/mg protein. No specific and saturable binding was detected in fractions enriched in the P2, P3, or S3 fractions.

Table II.

Subcellular fractionation studies utilizing [^{3}H]-[Met^5]-enkephalin and PANC-1 human pancreatic cells.

Fraction	Cellular Composition	K_d, nM	B_{max} (fmol/mg protein)
P1	Nuclear	2.3 ± 0.5	21.8 ± 4.8
P2	Membranes	NB	NB
P3	Microsomal	NB	NB
S3	Soluble	NB	NB

Values represent means ± SE for 3 independent assays. K_d, dissociation constant; B_{max}, binding capacity. NB = no specific or saturable binding.

Stage of growth on PANC-1 cells

PANC-1 cells grown for 3 to 4 days (log phase), 6-7 (confluent), or 9 days (post-confluent) were assayed for binding to radiolabeled [Met^5]-enkephalin (**Table III**). The length of time in culture had no effect on the affinity of [^{3}H]-[Met^5]-enkephalin. However, in contrast to post-confluent cells, the binding capacity of radiolabeled [Met^5]-enkephalin was reduced by 42% and 78% from the log-phase and confluent cultures, respectively.

Table III.

Binding of [^{3}H]-[Met^5]-enkephalin and PANC-1 human pancreatic cells at different stages of growth.

	Days in culture	K_d, nM	B_{max} (fmol/mg protein)
Log phase	3	4.2 ± 2.1	20.7 ± 1 2
Confluent	5	5.3 ± 0.0	7.8 ± 0.0**
Post-confluent	7-8	13.1 ± 1.1	35.6 ± 0.3**

Values represent means ± SE for 3 independent assays. K_d, dissociation constant; B_{max}, binding capacity. **Significantly different from log phase at $p<0.01$.

Ubiquity of OGFr in pancreatic cancer cell lines and xenografts

To examine for the presence of OGFr in a variety of pancreatic adenocarcinoma, saturation binding isotherms were performed on nuclear homogenates obtained from log phase BxPc-3, MIA PaCa-2, Capan-1, and Capan-2 cells (**Table IV**). The K_d ranged from 1.2 to 10.8 nM, and the B_{max} ranged from 11.9 to 25.5 fmol/mg protein.

Xenografts of human pancreatic cancer were performed according to earlier procedures (Zagon et al., *Cancer letters* 112: 167-175, 1997). In brief, 200,000 log-phase Capan-1 were injected subcutaneously into 4-week-old athymic nude mice (Charles River Laboratories, Wilmington, MA). Tumor tissue was harvested 40 days after cell inoculation. Xenografts of Capan-1 growing in nude mice revealed a K_d of 2.9 ± 1.5 nM and a B_{max} of 4.2 ± 1.1 fmol/mg protein.

Table IV.

Specific binding for [^{3}H]-[Met^5]-enkephalin to nuclear fractions of a variety of human pancreatic cancer cell lines.

Cell Line	State of Differentiation	K_d, nM	B_{max}, fmol/mg
MIA PaCA-2	Moderately to well	10.8 ± 5.5	11.9 ± 1.5
Capan-1	Metastatic to liver	6.5 ± 4.1	22.7 ± 9.5
BxPC-3	Moderately to well	1.2 ± 0.3	25.5 ± 9.7
PANC-1	Poor	5.1 ± 1.4	28.9 ± 6.2

Values represent means ± SE for at least 3 independent assays. K_d, dissociation constant; B_{max}, binding capacity.

OGFr in neoplastic and normal pancreatic tissues

Tumor specimens were obtained at the time of pancreatic resection from four females ranging in age from 52- to 66-yrs, and a 67-yr-old male. Normal tissues (as determined from histological assessment) were obtained from two females (61- and 66-yr-old) and a male (55-yr-old). In the case of the normal samples from the two females, the specimens were adjacent to the tumor tissue. All human tissue specimens were collected with the approval of the Institutional Review Board, Human Subjects Protection Office, The Pennsylvania State University College of Medicine. Samples were collected and frozen in liquid nitrogen within 1 hr of surgery. Receptor binding assays were performed as stated above.

Human pancreatic cancer obtained at resection showed a mean K_d of 2.1 ± 1.0 nM and a B_{max} of 6.6 ± 1.2 fmol/mg protein for the specimens harvested from 3 patients; in 3 specimens, non-specific and non-saturable binding were recorded. Of the 3 normal specimens of human pancreas, a mean K_d of 9.6 ± 2.9 and B_{max} of 46.7 ± 27.8 fmol/mg protein was detected.

Example 8

Decreased OGFr Number in Head and Neck Squamous Cell Carcinoma Compared to Normal Mucosa

To compare the ζ opioid receptor in SCCHN versus normal mucosa, pharmacological binding assays utilizing enriched nuclear preparations of human tissue obtained from surgery and [^{3}H]-[Met^5]-enkephalin were conducted. Specific and saturable binding of a one-site receptor model was confirmed. Human tissue samples were representative of a variety of squamous cell carcinomas, including specimens from the oral cavity-larynx, and regional metastases. Normal mucosal specimens were obtained during uvu-lopalatopharyngoplasty. Binding capacity (B_{max}) of the radioactive ligand, and index of receptor number, was 80.8.±32.8 fmol/mg protein for normal epithelium in comparison to a 6-fold less B_{max} of 13.6±1.9 fmol/mg protein for neoplastic specimens; these values differed significantly ($p<0.01$). No differences in binding were noted among the carcinogenic tissues assayed. Specific affinity (k_α) values were comparable between normal and neoplastic tissues being 11.6±2.5 and 4.1±0.4 nM respectively. These data indicate that opioid growth factor receptor levels are reduced in human SCCHN when compared to normal mucosa. Nonetheless, the receptors are present and capable of binding ligand and with normal affinity suggesting that supplementation with exogenous OGF might be inhibitory to cancer cells.

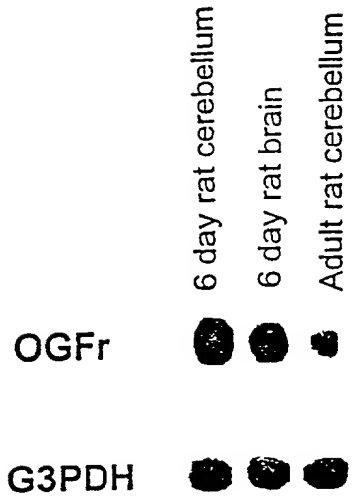
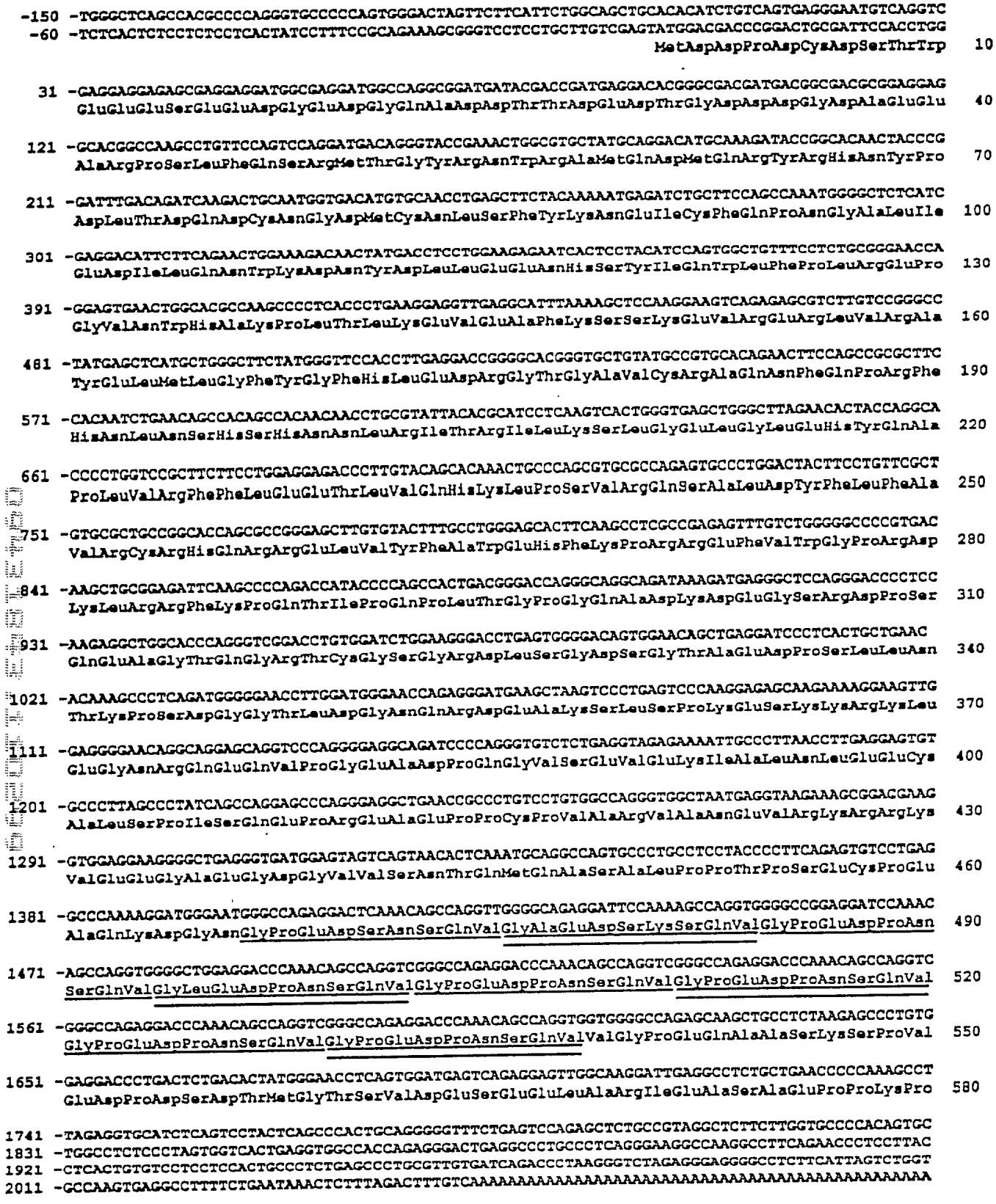

6 day rat cerebellum
6 day rat brain
Adult rat cerebellum
OGFr
G3PDH

Figure 1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GlyProGluAspProAsnSerGlnValGlyProGluAspProAsnSerGlnValValGlyProGluGlnAlaAlaSerLysSerProVal 550
1651 -GAGGACCCTGACTCTGACACTATGGGAACCTCAGTGGATGAGTCAGAGGAGTTGGCAAGGATTGAGGCCTCTGCTGAACCCCCAAAGCCT
GluAspProAspSerAspThrMetGlyThrSerValAspGluSerGluGluLeuAlaArgIleGluAlaSerAlaGluProProLysPro 580
1741 -TAGAGGTGCATCTCAGTCCTACTCAGCCCACTGCAGGGGGTTTCTGAGTCCAGAGCTCTGCCGTAGGCTCTTCTTGGTGCCCCACAGTGC
1831 -TGGCCTCTCCCTAGTGGTCACTGAGGTGGCCACCAGAGGGACTGAGGCCCTGCCCTCAGGGAAGGCCAAGGCCTTCAGAACCCTCCTTAC
1921 -CTCACTGTGTCCTCCTCCACTGCCCTCTGAGCCCTGCGTTGTGATCAGACCCTAAGGGTCTAGAGGGAGGGGCCTCTTCATTAGTCTGGT
2011 -GCCAAGTGAGGCCTTTTCTGAATAAACTCTTTAGACTTTGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 2

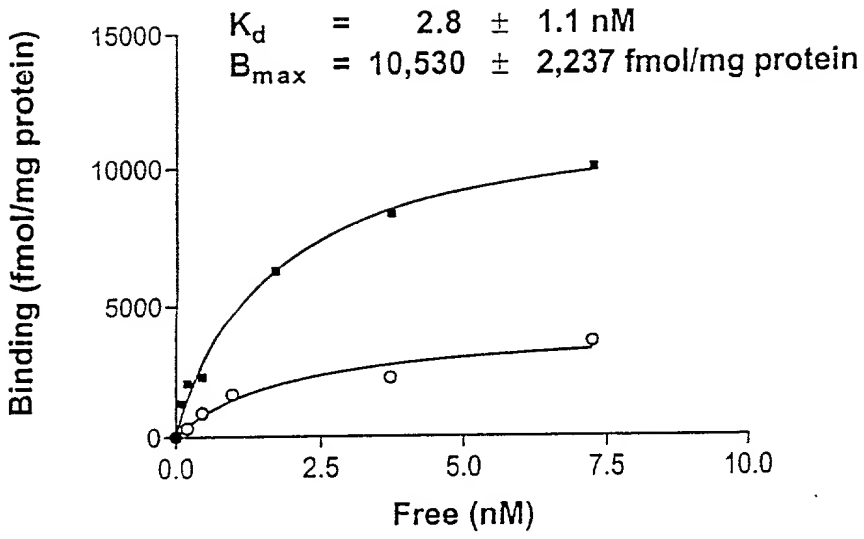

K_d = 2.8 ± 1.1 nM
B_{max} = 10,530 ± 2,237 fmol/mg protein
Binding (fmol/mg protein)
15000
10000
5000
0
0.0
2.5
5.0
7.5
10.0
Free (nM)

Figure 3

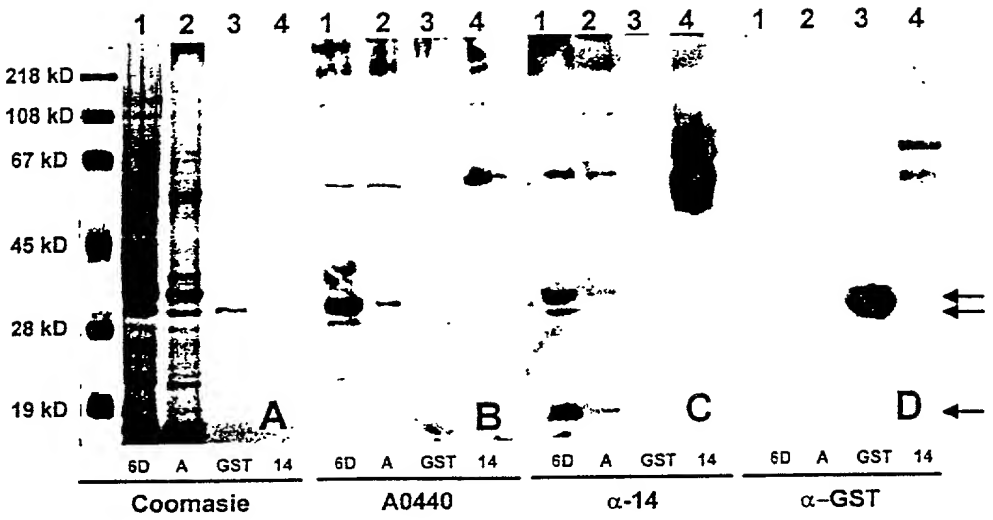

1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
218 kD
108 kD
67 kD
45 kD
28 kD
19 kD
A
B
C
D
6D A GST 14 6D A GST 14 6D A GST 14 6D A GST 14
Coomasie
A0440
α-14
α-GST

Figure 4

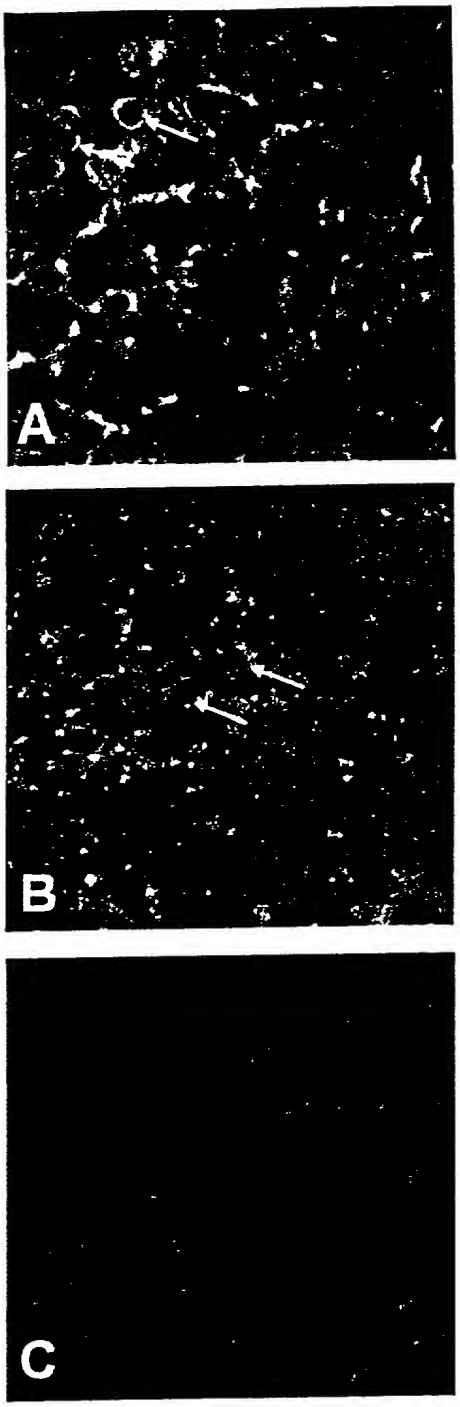


Figure 5

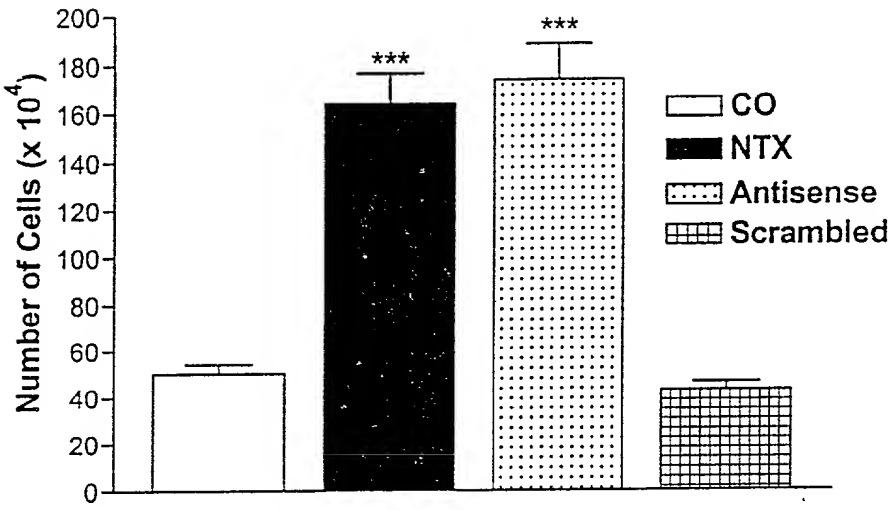

Number of Cells (x 10^4)
200
180
160
140
120
100
80
60
40
20
0
***
***
CO
NTX
Antisense
Scrambled

Figure 6

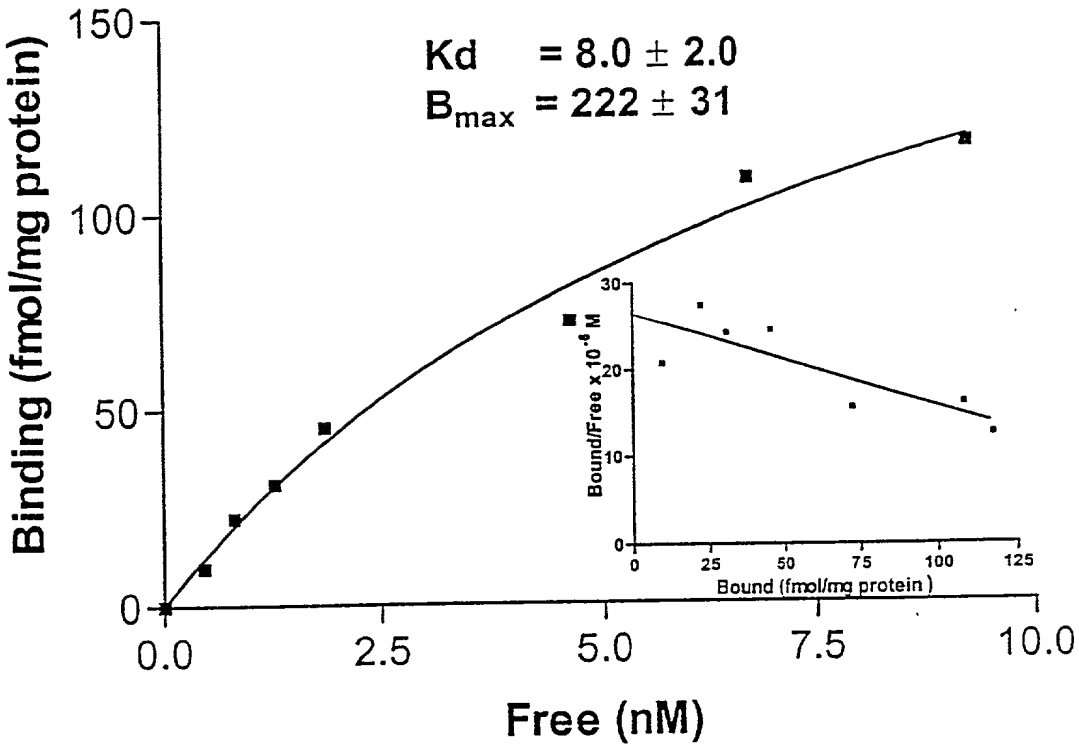
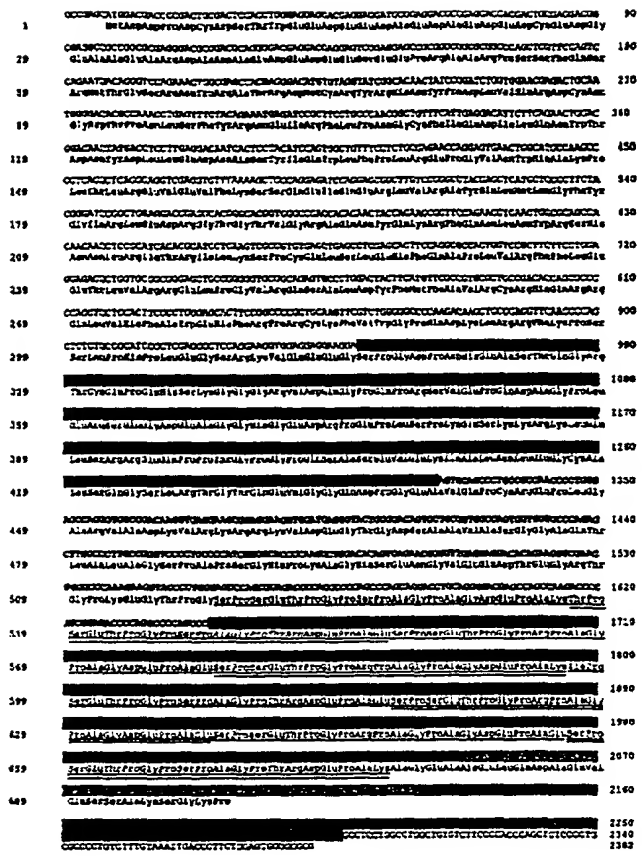
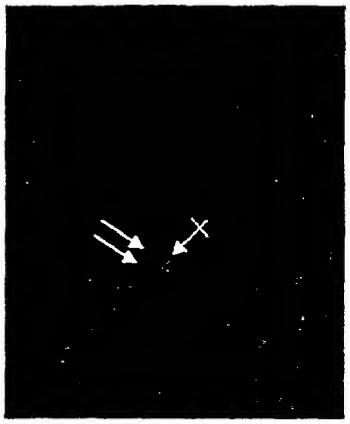

Kd = 8.0 ± 2.0
Bmax = 222 ± 31
Binding (fmol/mg protein)
150
100
50
0
0.0
2.5
5.0
7.5
10.0
Free (nM)
Bound/Free x 10⁻³ M
30
20
10
0
0
25
50
75
100
125
Bound (fmol/mg protein)

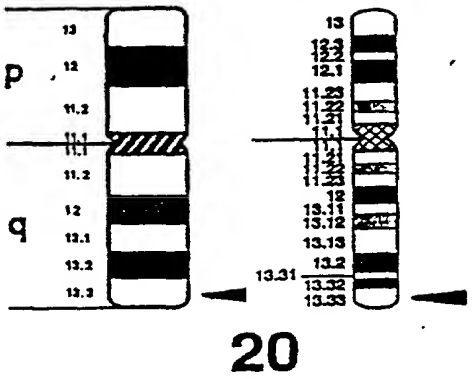
Figure 7

A.

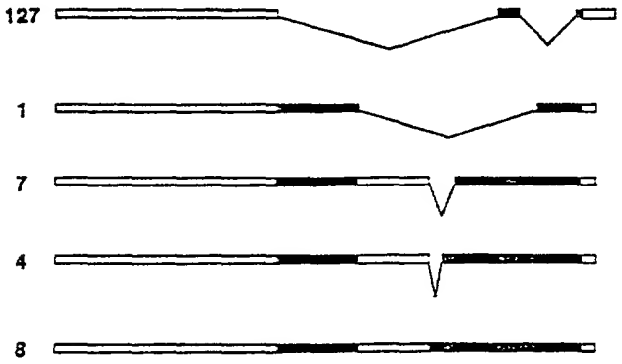


E.

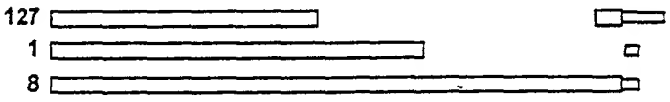




B.



C.



D.

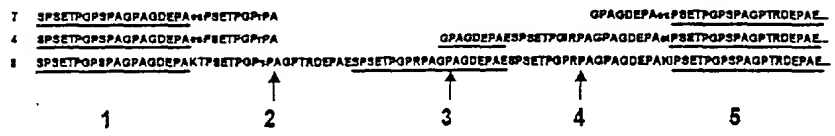


Figure 8

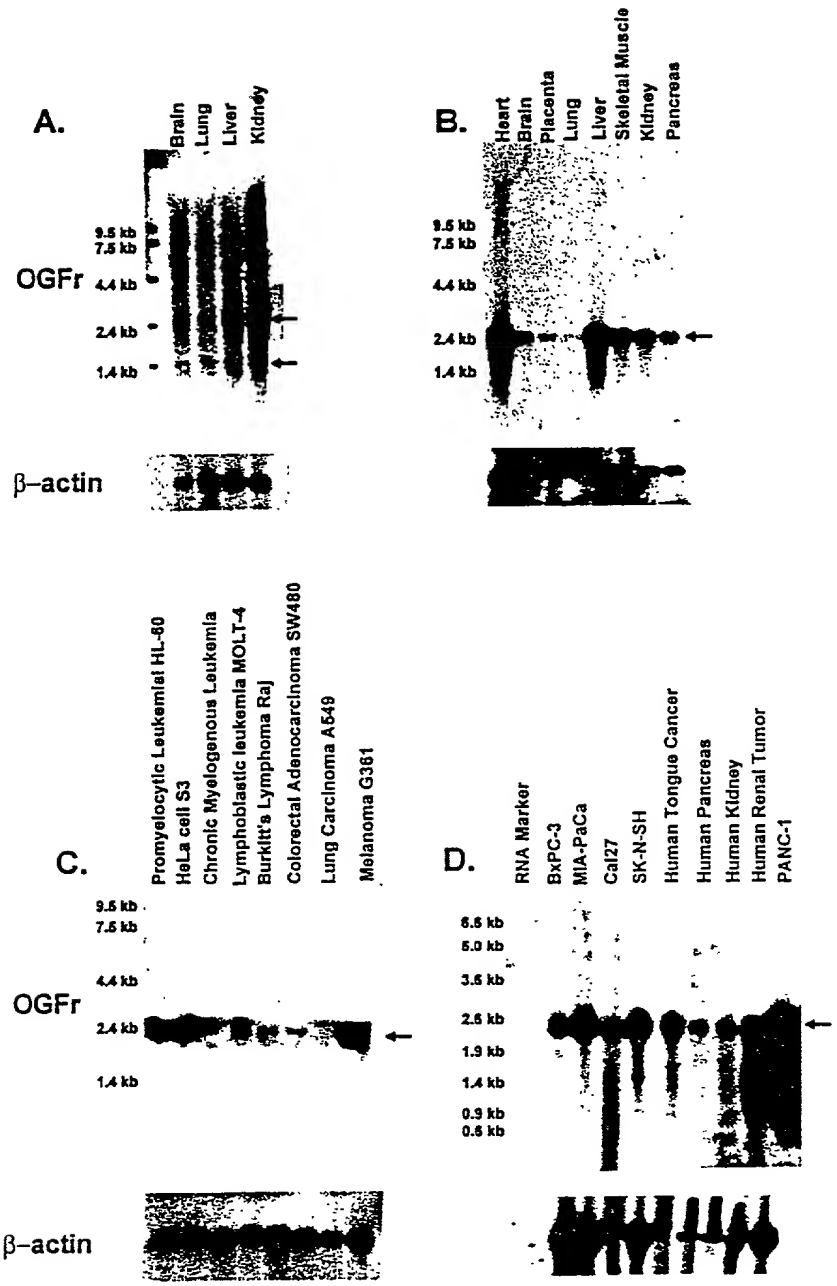

A.
Brain
Lung
Liver
Kidney
9.5 kb
7.5 kb
OGFr
4.4 kb
2.4 kb
1.4 kb
β–actin
B.
Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
9.5 kb
7.5 kb
4.4 kb
2.4 kb
1.4 kb
C.
Promyelocytic Leukemia HL-60
HeLa cell S3
Chronic Myelogenous Leukemia
Lymphoblastic leukemia MOLT-4
Burkitt's Lymphoma Raj
Colorectal Adenocarcinoma SW480
Lung Carcinoma A549
Melanoma G361
9.5 kb
7.5 kb
4.4 kb
OGFr
2.4 kb
1.4 kb
β–actin
D.
RNA Marker
BxPC-3
MIA-PaCa
Cal27
SK-N-SH
Human Tongue Cancer
Human Pancreas
Human Kidney
Human Renal Tumor
PANC-1
6.6 kb
5.0 kb
3.6 kb
2.6 kb
1.9 kb
1.4 kb
0.9 kb
0.6 kb

Figure 9

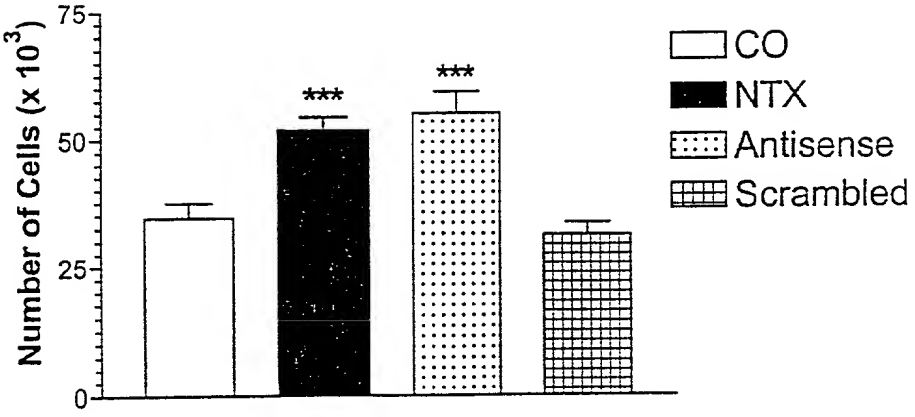


Figure 10

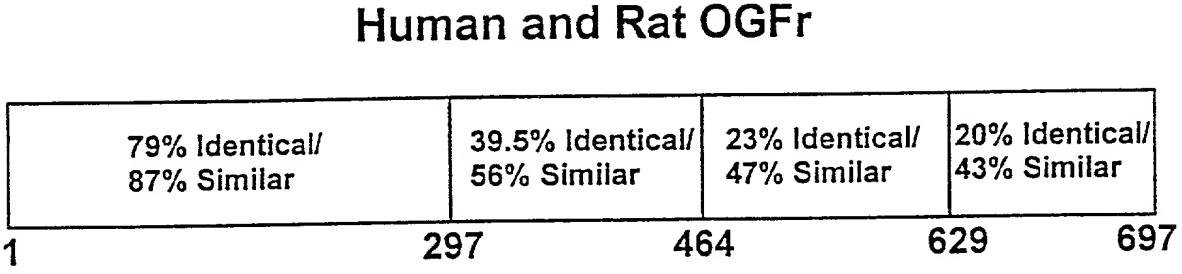

Human and Rat OGFr
79% Identical/
87% Similar
39.5% Identical/
56% Similar
23% Identical/
47% Similar
20% Identical/
43% Similar
1
297
464
629
697
Amino Acid Number

Figure 11

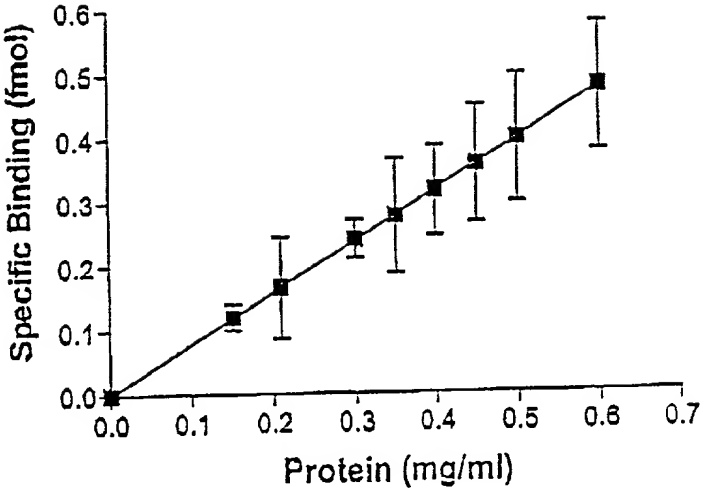

Specific Binding (fmol)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Protein (mg/ml)

Figure 12

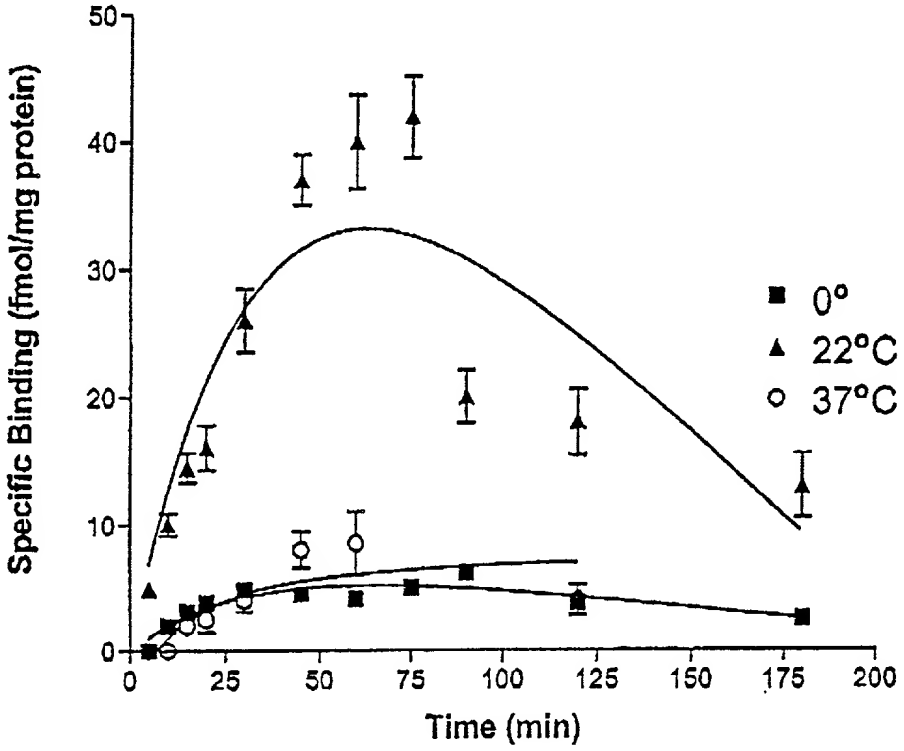

Specific Binding (fmol/mg protein)
50
40
30
20
10
0
0
25
50
75
100
125
150
175
200
Time (min)
0°
22°C
37°C

Figure 13

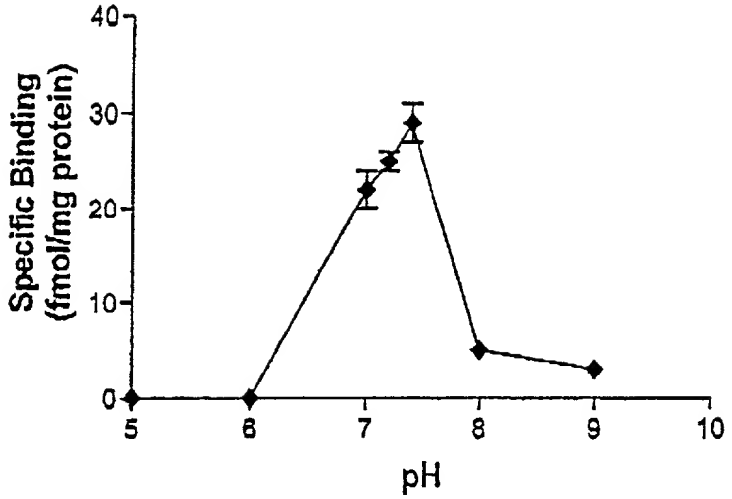


Figure 14

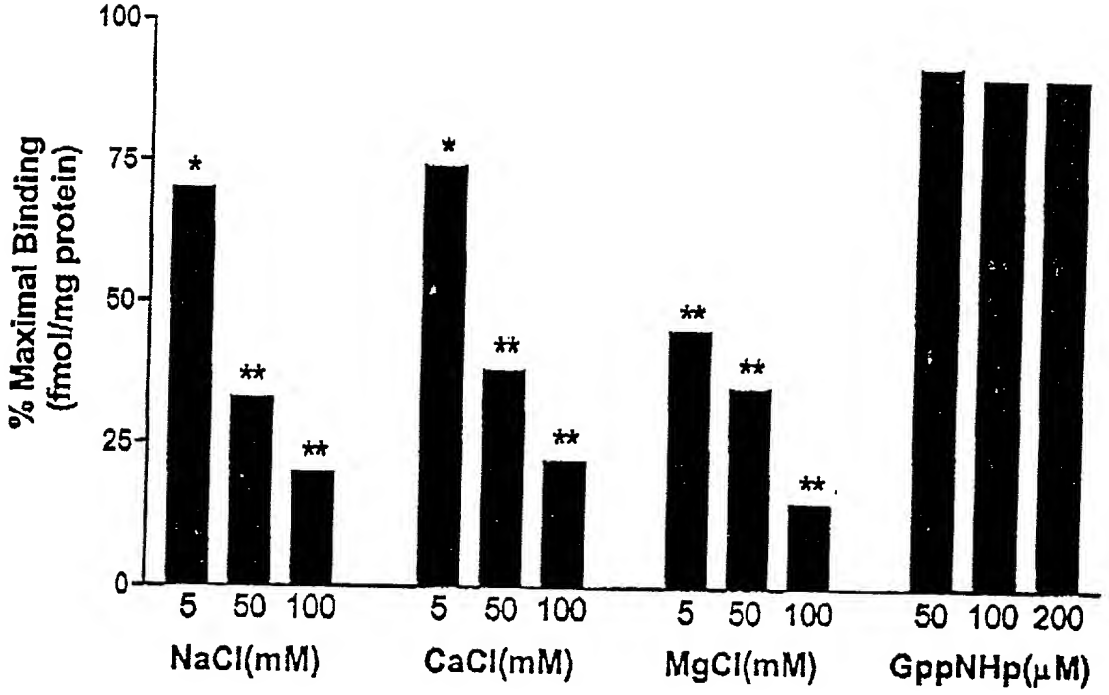

% Maximal Binding
(fmol/mg protein)
100
75
50
25
0
*
**
**
*
**
**
**
**
**
5 50 100
NaCl(mM)
5 50 100
CaCl(mM)
5 50 100
MgCl(mM)
50 100 200
GppNHp(μM)

Figure 15

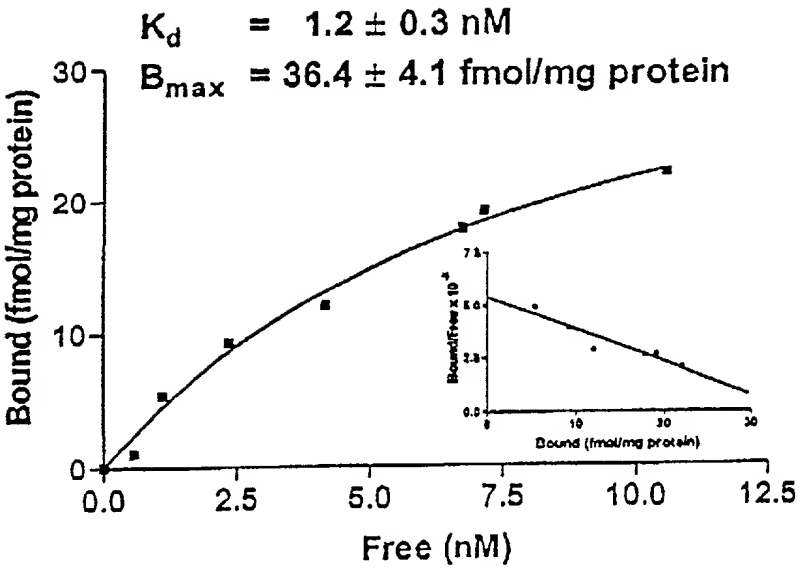

K_d = 1.2 ± 0.3 nM
B_{max} = 36.4 ± 4.1 fmol/mg protein
Bound (fmol/mg protein)
30
20
10
0
0.0
2.5
5.0
7.5
10.0
12.5
Free (nM)

Figure 16

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

"NOVEL NUCLEIC ACID MOLECULES ENCODING OPIOID GROWTH FACTOR RECEPTORS"

the specification of which

(check one) [XXXX] is attached hereto.
[ ] was filed on _____ as United States Application Number _____
or PCT International Application Number _____ and was amended on
_____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.
I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	[ ]
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	[ ]

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

60/106,879 (Application Number)	11/03/98 (Filing Date)
_____ (Application Number)	_____ (Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

_____ (Application Number)	_____ (Filing Date)	_____ (Status-patented, pending, abandoned)
_____ (Application Number)	_____ (Filing Date)	_____ (Status-patented, pending, abandoned)

DECLARATION FOR PATENT APPLICATION

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Thomas J. Monahan (Reg. No. 29,835); Frank S. DiGiglio (Reg. No. 31,346)

Address all telephone calls to Thomas J. Monahan at telephone no. 814-865-6279.

Address all correspondence to Intellectual Property Office
The Pennsylvania State University
113 Technology Center
University Park, PA 16802

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name): IAN S. ZAGON

Inventor's signature: _____ Date: _____
Residence: Hummelstown, Pennsylvania Citizenship: U.S.A.
Post Office Address: 589 Cook Court, Hummelstown, Pennsylvania 17036

Full name of second joint inventor, if any (given name, family name): PATRICIA J. McLAUGHLIN

Second Inventor's signature: _____ Date: _____
Residence: Harrisburg, Pennsylvania Citizenship: U.S.A.
Post Office Address: 5535 Partridge Court, Harrisburg, Pennsylvania 17111

Full name of third joint inventor, if any (given name, family name): MICHAEL F. VERDERAME

Third Inventor's signature: _____ Date: _____
Residence: Hershey, Pennsylvania Citizenship: U.S.A.
Post Office Address: 1882 Sand Hill Road, Hershey, Pennsylvania 17033

Sequence Listing

SEQ ID		
1	rat OGFr cDNA	
2	rat OGFr protein	
3	rat OGFr cDNA partial (clone 14)	
4	human OGFr cDNA (Provisional)	
5	human OGFr splice version 8, cDNA	
6	human OGFr splice version 8, protein	
7	human OGFr splice version 1, cDNA	
8	human OGFr splice version 1, protein	
9	human OGFr splice version 4, cDNA	
10	human OGFr splice version 4, protein	
11	human OGFr splice version 7, cDNA	
12	human OGFr splice version 7, protein	
13	human OGFr splice version 127, cDNA	
14	human OGFr splice version 127, protein	
15	rat OGFr antisense	5'-GACTCAGGGACTTAGCTTCATCC-3'
16	scrambled	5'-ATAGATACTACGCCGGCTGTCCT-3'
17	human OGFr antisense	5'-GGTCGTCCATGCTCGGCTAGAAT-3'
18	scrambled	5'-GTGCAGTGCAATGCTCTCCGTGA-3'

SEQ ID NO: 1 -- Rat Opioid Growth Factor Receptor cDNA sequence

```
TGGGCTCAGCCACGCCCCAGGGTGCCCCCAGTGGGACTAGTTCTTCATTCTGGCAGCTGCACACATCTGTCAGTGAGGGAATGTCAGGTC   90
TCTCACTCTCCTCTCCTCACTATCCTTTCCGCAGAAAGCGGGTCCTCCTGCTTGTCGAGTATGGACGACCCGGACTGCGATTCCACCTGG  180
GAGGAGGAGAGCGAGGAGGATGGCGAGGATGGCCAGGCGGATGATACGACCGATGAGGACACGGGCGACGATGACGGCGACGCGGAGGAG  270
GCACGGCCAAGCCTGTTCCAGTCCAGGATGACAGGGTACCGAAACTGGCGTGCTATGCAGGACATGCAAAGATACCGGCACAACTACCCG  360
GATTTGACAGATCAAGACTGCAATGGTGACATGTGCAACCTGAGCTTCTACAAAAATGAGATCTGCTTCCAGCCAAATGGGGCTCTCATC  450
GAGGACATTCTTCAGAACTGGAAAGACAACTATGACCTCCTGGAAGAGAATCACTCCTACATCCAGTGGCTGTTTCCTCTGCGGGAACCA  540
GGAGTGAACTGGCACGCCAAGCCCCTCACCCTGAAGGAGGTTGAGGCATTTAAAAGCTCCAAGGAAGTCAGAGAGCGTCTTGTCCGGGCC  630
TATGAGCTCATGCTGGGCTTCTATGGGTTCCACCTTGAGGACCGGGGCACGGGTGCTGTATGCCGTGCACAGAACTTCCAGCCGCGCTTC  720
CACAATCTGAACAGCCACAGCCACAACAACCTGCGTATTACACGCATCCTCAAGTCACTGGGTGAGCTGGGCTTAGAACACTACCAGGCA  810
CCCCTGGTCCGCTTCTTCCTGGAGGAGACCCTTGTACAGCACAAACTGCCCAGCGTGCGCCAGAGTGCCCTGGACTACTTCCTGTTCGCT  900
GTGCGCTGCCGGCACCAGCGCCGGGAGCTTGTGTACTTTGCCTGGGAGCACTTCAAGCCTCGCCGAGAGTTTGTCTGGGGGCCCCGTGAC  990
AAGCTGCGGAGATTCAAGCCCCAGACCATACCCCAGCCACTGACGGGACCAGGGCAGGCAGATAAAGATGAGGGCTCCAGGGACCCCTCC 1080
CAAGAGGCTGGCACCCAGGGTCGGACCTGTGGATCTGGAAGGGACCTGAGTGGGGACAGTGGAACAGCTGAGGATCCCTCACTGCTGAAC 1170
ACAAAGCCCTCAGATGGGGGAACCTTGGATGGGAACCAGAGGGATGAAGCTAAGTCCCTGAGTCCCAAGGAGAGCAAGAAAAGGAAGTTG 1260
GAGGGGAACAGGCAGGAGCAGGTCCCAGGGGAGGCAGATCCCCAGGGTGTCTCTGAGGTAGAGAAAATTGCCCTTAACCTTGAGGAGTGT 1350
GCCCTTAGCCCTATCAGCCAGGAGCCCAGGGAGGCTGAACCGCCCTGTCCTGTGGCCAGGGTGGCTAATGAGGTAAGAAAGCGGAGGAAG 1440
GTGGAGGAAGGGGCTGAGGGTGATGGAGTAGTCAGTAACACTCAAATGCAGGCCAGTGCCCTGCCTCCTACCCCTTCAGAGTGTCCTGAG 1530
GCCCAAAAGGATGGGAATGGGCCAGAGGACTCAAACAGCCAGGTTGGGGCAGAGGATTCCAAAAGCCAGGTGGGGCCGGAGGATCCAAAC 1620
AGCCAGGTGGGGCTGGAGGACCCAAACAGCCAGGTCGGGCCAGAGGACCCAAACAGCCAGGTCGGGCCAGAGGACCCAAACAGCCAGGTC 1710
GGGCCAGAGGACCCAAACAGCCAGGTCGGGCCAGAGGACCCAAACAGCCAGGTGGTGGGGCCAGAGCAAGCTGCCTCTAAGAGCCCTGTG 1800
GAGGACCCTGACTCTGACACTATGGGAACCTCAGTGGATGAGTCAGAGGAGTTGGCAAGGATTGAGGCCTCTGCTGAACCCCCAAAGCCT 1890
TAGAGGTGCATCTCAGTCCTACTCAGCCCACTGCAGGGGGTTTCTGAGTCCAGAGCTCTGCCGTAGGCTCTTCTTGGTGCCCCACAGTGC 1980
TGGCCTCTCCCTAGTGGTCACTGAGGTGGCCACCAGAGGGACTGAGGCCCTGCCCTCAGGGAAGGCCAAGGCCTTCAGAACCCTCCTTAC 2070
CTCACTGTGTCCTCCTCCACTGCCCTCTGAGCCCTGCGTTGTGATCAGACCCTAAGGGTCTAGAGGGAGGGGCCTCTTCATTAGTCTGGT 2160
GCCAAGTGAGGCCTTTTCTGAATAAACTCTTTAGACTTTGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2250
```

Initiator AUG at 151-153

Terminator TAG at 1891-1983

Open reading frame 151 (AUGGACGAC...) to 1890 (... AAGCCT)

SEQ ID NO:2 -- Rat Opioid Growth Factor Receptor

```
   1  MDDPDCDSTW EEESEEDGED GQADDTTDED TGDDDGDAEE ARPSLFQSRM
  51  TGYRNWRAMQ DMQRYRHNYP DLTDQDCNGD MCNLSFYKNE ICFQPNGALI
 101  EDILQNWKDN YDLLEENHSY IQWLFPLREP GVNWHAKPLT LKEVEAFKSS
 151  KEVRERLVRA YELMLGFYGF HLEDRGTGAV CRAQNFQPRF HNLNSHSHNN
 201  LRITRILKSL GELGLEHYQA PLVRFFLEET LVQHKLPSVR QSALDYFLFA
```

```
 251  VRCRHQRREL VYFAWEHFKP RREFVWGPRD KLRRFKPQTI PQPLTGPGQA
 301  DKDEGSRDPS QEAGTQGRTC GSGRDLSGDS GTAEDPSLLN TKPSDGGTLD
 351  GNQRDEAKSL SPKESKKRKL EGNRQEQVPG EADPQGVSEV EKIALNLEEC
 401  ALSPISQEPR EAEPPCPVAR VANEVRKRRK VEEGAEGDGV VSNTQMQASA
 451  LPPTPSECPE AQKDGNGPED SNSQVGAEDS KSQVGPEDPN SQVGLEDPNS
 501  QVGPEDPNSQ VGPEDPNSQV GPEDPNSQVG PEDPNSQVVG PEQAASKSPV
 551  EDPDSDTMGT SVDESEELAR IEASAEPPKP
```

SEQ ID NO:3 -- Rat OGFr, partial cDNA sequence, clone 14

```
   1  CATTGGGCCG ACGTCGCATG CTCCTCTAGA CTCGAGGAAT TCGGGCCCCA
  51  GGGTGTCTCT GAGGTAGAGA AAATTGCCCT TAACCTTGAG GAGTGTGCCC
 101  TTAGCCCTAT CAGCCAGGAG CCCAGGGAGG STGAACCGCC CTGTCCTGTG
 151  GCCAGGGTGG CTANAATGAG GTAAGAAANG CGGNAGGAAG GTGGAGGAAG
 201  GGGCTGAGGG TGNATGGAGT AGTCAGTAAC ACTYAAATGN CAGGCCAGTG
 251  CCCTGCCTCC TACCCCTTCA GAGTGTCCTG AGGCCCAAAA GGATGGGAAT
 301  GGGCCAGAGG ACTCAAACAG CCAGGTTGGG GCAGAGGATT CCAAAAGCCA
 351  GGTGGGGCCG GAGGATCCAA ACAGCCAGGT GGGGCTGGAG GACCCAAACA
 401  GCCAGGTCGG GCCAGAGGAC CCAAACAGCC AGGTCGGGCC AGAGGACCCA
 451  AACAGCCAGG TCGGGCCAGA GGACCCAAAC AGCCAGGTCG GGCCAGAGGA
 501  CCCAAACAGC CAGGTGGTGG GGCCAGAGCA AGCTGCCTCT AAGAGCCCTG
 551  TGGANGGACC CTGACTCTGA CACTATGGGA ACCTCAGTGG ATGAGTCAGA
 601  GGAGTTGGCA AGGATTGAGG CNTYTGCTGA ACCCCCAAAG CCTTAGAGGT
 651  GCATTTCAGT CCTACTCAGC CCACTGCAGG GGGTTTCTGA GTCCAGAGCT
 701  CTGCCGTAGG CTCTTCTTGG TGCCCCACAG TGCTGGCCTC TCCCTASTGG
 751  TCACTGAGGT GGCCACCAGA GGGACTGAGG CCCTGCCCTC AGGGAAGGCC
 801  AAGGCCTTCA GAACCCTCCT TACCTCACTG TGTCCTCCTC CACTGCCCTC
 851  TGAGCCCTGC GTTGTGATCA GACCCTAAGG GTCTAGAGGG AGGGGCCTCT
 901  TCATTAGTCT GGTGCCAAGT GAGGCCTTTT CTGAATAAAC TCTTTAGACT
 951  TTGTCAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAA
```

SEQ ID NO:4 -- Human OGF receptor cDNA; spliced form A

Length: 2290

```
   1 TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
  51 CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
 101 AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
 151 GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
 201 CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
 251 GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
 301 GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
 351 CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC
 401 TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
 451 CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
 501 GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
 551 TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
 601 GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
 651 CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
 701 TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
 751 ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
 801 CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
 851 TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
 901 GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCCCATC CGCTCGAGGG
 951 CTCCAGGAAG GTGGAGGAGG AAGGAAGCCC CGGGGACCCC GACCACGAGG
1001 CCAGCACCCA GGGTCGGACC TGTGGGCCAG AGCATAGCAA GGGTGGGGGC
1051 AGGGTGGACG AGGGGCCCCA GCCACGGAGC GTGGAGCCCC AGGATGCGGG
1101 ACCCCTGGAG AGGAGCCAGG GGGATGAGGC AGGGGGCCAC GGGGAAGATA
1151 GGCCGGAGCC CTTAAGCCCC AAAGAGAGCA AGAAGAGGAA GCTGGAGCTG
1201 AGCCGGCGGG AGCAGCCGCC CACAGAGCCA GGCCCTCAGA GTGCCTCAGA
1251 GGTGGAGAAG ATCGCTCTGA ATTTGGAGGG GTGTGCCCTC AGCCAGGGCA
1301 GCCTCAGGAC GGGGACCCAG GAAGTGGGCG GTCAGGACCC TGGGGAGGCA
1351 GTGCAGCCCT GCCGCCAACC CCTGGGAGCC AGGGTGGCCG ACAAGGTGAG
1401 GAAGCGGAGG AAGGTGGATG AGGGTGCTGG GGACAGTGCT GCGGTGGCCA
1451 GTGGTGGTGC CCAGACCTTG GCCCTTGCCG GGTCCCCTGC CCCATCGGGG
1501 CACCCCAAGG CTGGACACAG TGAGAACGGG GTTGAGGAGG ACACAGAAGG
1551 TCGAACGGGG CCCAAAGAAG GTACCCCTGG GAGCCCATCG GAGACCCCAG
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1601 GCCCCAGCCC AGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG
1651 GAGACCCCAG GCCCCCGCCC GGCAGGACCT GCAGGGGACG AGCCAGCCGA
1701 GAGCCCATCG GAGACCCCAG GCCCCAGCCC GGCAGGACCT ACAAGGGATG
1751 AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC GGCAGGACCT
1801 GCAGGGGACG AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC
1851 GGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG GAGACCCCAG
1901 GCCCCAGCCC GGCAGGACCT ACAAGGGATG AGCCAGCCAA GGCGGGGGAG
1951 GCAGCAGAGT TGCAGKACGC AGAGGTGGAG TCTTCTGCCA AGTCTGGGAA
2001 GCCTTAAGGA AAGGAGTGCC CGTCGGCGTC TTGGTCCTCC TGTCCCTGCT
2051 GCAGGGGCTG GGGCCTCCGG AGCTTGCTGC GGGCTCCCCT CAGGCTCTGC
2101 TTCGTGACCC GTGACCCATG ACCCACAGTG CTGGCCTCCT GTGGGGCCAC
2151 TATAGCARSC ACCAGAAGCC GCGAGGCCCT CAGGGAAGCC CAAGGCCTGC
2201 AGAAGCCTCC TGGCCTGGCT GTGTCTTCCC CACCCAGCTC TCCCCTGCGC
2251 CCCTGTCTTT GTAAATTGAC CCTTCTGGAG TGGGGGGCGG

Letter"S", "R", or "K" was used in positions where there was a 50-50 split on the consensus sequences. S = C or G; R = A or G; K = T or G.
Inititator AUG 34-36
Terminator TAA 2005-2007
Open reading frame: 34 (AUGGACGAC...) to 2004 (...GGGAAGCCT)

SEQ ID NO: 5 -- Human Opioid Growth Factor Receptor cDNA, spliced version 8

1 TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
51 CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
101 AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
151 GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
201 CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
251 GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
301 GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
351 CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC

401 TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
451 CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
501 GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
551 TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
601 GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
651 CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
701 TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
751 ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
801 CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
851 TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
901 GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCGCATC CGCTCGAGGG
951 CTCCAGGAAG GTGGAGGAGG AAGGAAGCCC CGGGGACCCC GACCACGAGG
1001 CCAGCACCCA GGGTCGGACC TGTGAGCCAG AGCATAGCAA GGGTGGGGGC
1051 AGGGTGGACG AGGGGCCCCA GCCACGGAGC GTGGAGCCCC AGGATGCGGG
1101 ACCCCTGGAG AGGAGCCAGG GGGATGAGGC AGGGGGCCAC GGGGAAGATA
1151 GGCCGGAGCC CTTAAGCCCC AAAGAGAGCA AGAAGAGGAA GCTGGAGCTG
1201 AGCCGGCGGG AGCAGCCGCC CACAGGGCCA GGCCCTCAGA GTGCCTCAGA
1251 GGTGGAGAAG ATCGCTCTGA ATTTGGAGGG GTGTGCCCTC AGCCAGGGCA
1301 GCCTCAGGAC GGGGACCCAG GAAGTGGGCG GTCAGGACCC TGGGGAGGCA
1351 GTGCAGCCCT GCCGCCAACC CCTGGGAGCC AGGGTGGCCG ACAAGGTGAG
1401 GAAGCGGAGG AAGGTGGATG AGGGTACTGG GGACAGTGCT GCGGTGGCCA
1451 GTGGTGGTGC CCAGACCTTG GCCCTTGCCG GGTCCCCTGC CCCATCGGGG
1501 CACCCCAAGG CTGGACACAG TGAGAACGGG GTTGAGGAGG ACACAGAAGG
1551 TCGAACGGGG CCCAAAGAAG GTACCCCTGG GAGCCCATCG GAGACCCCAG
1601 GCCCCAGCCC AGCAGGACCT GCAGGGGACG AGCCAGCCAA GACCCCATCG
1651 GAGACCCCAG GCCCCAGCCC GGCAGGACCT ACAAGGGATG AGCCAGCCGA
1701 GAGCCCATCG GAGACCCCAG GCCCCCGCCC GGCAGGACCT GCAGGGGACG
1751 AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC GGCAGGACCT
1801 GCAGGGGACG AGCCAGCCAA GATCCCATCG GAGACCCCAG GCCCCAGCCC
1851 GGCAGGACCT ACAAGGGATG AGCCAGCCGA GAGCCCATCG GAGACCCCAG
1901 GCCCCCGCCC GGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG
1951 GAGACCCCAG GCCCCCGCCC GGCAGGACCT GCAGGGGACG AGCCAGCCGA

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2001  GAGCCCATCG GAGACCCCAG GCCCCAGCCC GGCAGGACCT ACAAGGGATG
2051  AGCCAGCCAA GGCGGGGGAG GCAGCAGAGT TGCAGGACGC AGAGGTGGAG
2101  TCTTCTGCCA AGTCTGGGAA GCCTTAAGGA AAGGAGTGCC CGTCGGCGTC
2151  TTGGTCCTCC TGTCCCTGCT GCAGGGGCTG GGGCCTCCGG AGCTGCTGCG
2201  GGCTCCCCTC AGGCTCTGCT TCGTGACCCG TGACCCATGA CCCACAGTGC
2251  TGGCCTCCTG TGGGGCCACT ATAGCAGCCA CCAGAAGCCG CGAGGCCCTC
2301  AGGGAAGCCC AAGGCCTGCA GAAGCCTCCT GGCCTGGCTG TGTCTTCCCC
2351  ACCCAGCTCT CCCCTGCGCC CCTGTCTTTG TAAATTGACC CTTCTGGAGT
2401  GGGGGGCG
```

SEQ ID NO: 6 -- Human Opioid Growth Factor Receptor protein, from spliced cDNA version 8

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MDDPDCDSTWEEDEEDAEDAEDEDCEDGEAAGARDADAGDEDEESEEPRAARPSSFQSRM   60
TGSRNWRATRDMCRYRHNYPDLVERDCNGDTPNLSFYRNEIRFLPNGCFIEDILQNWTDN  120
YDLLEDNHSYIQWLFPLREPGVNWHAKPLTLREVEVFKSSQEIQERLVRAYELMLGFYGI  180
RLEDRGTGTVGRAQNYQKRFQNLNWRSHNNLRITRILKSPCELSLEHFQAPLVRFFLEET  240
LVRRELPGVRQSALDYFMFAVRCRHQRRQLVHFAWEHFRPRCKFVWGPQDKLRRFKPSSL  300
PHPLEGSRKVEEEGSPGDPDHEASTQGRTCEPEHSKGGGRVDEGPQPRSVEPQDAGPLER  360
SQGDEAGGHGEDRPEPLSPKESKKRKLELSRREQPPTGPGPQSASEVEKIALNLEGCALS  420
QGSLRTGTQEVGGQDPGEAVQPCRQPLGARVADKVRKRRKVDEGTGDSAAVASGGAQTLA  480
LAGSPAPSGHPKAGHSENGVEEDTEGRTGPKEGTPGSPSETPGPSPAGPAGDEPAKTPSE  540
TPGPSPAGPTRDEPAESPSETPGPRPAGPAGDEPAESPSETPGPRPAGPAGDEPAKIPSE  600
TPGPSPAGPTRDEPAESPSETPGPRPAGPAGDEPAESPSETPGPRPAGPAGDEPAESPSE  660
TPGPSPAGPTRDEPAKAGEAAELQDAEVESSAKSGKP                          697
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SEQ ID NO: 7 -- Human OGFr cDNA, spliced version 1

```
   1  TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
  51  CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
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 101  AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
 151  GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
 201  CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
 251  GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
 301  GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
 351  CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC
 401  TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
 451  CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
 501  GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
 551  TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
 601  GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
 651  CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
 701  TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
 751  ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
 801  CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
 851  TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
 901  GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCGCATC CGCTCGAGGG
 951  CTCCAGGAAG GTGGAGGAGG AAGGAAGCCC CGGGGACCCC GACCACGAGG
1001  CCAGCACCCA GGGTCGGACC TGTGGGCCAG AGCATAGCAA GGGTGGGGGC
1051  AGGGTGGACG AGGGGCCCCA GCCACGGAGC GTGGAGCCCC AGGATGCGGG
1101  ACCCCTGGAG AGGAGCCAGG GGGATGAGGC AGGGGGCCAC GGGGAAGATA
1151  GGCCGGAGCC CTTAAGCCCC AAAGAGAGCA AGAAGAGGAA GCTGGAGCTG
1201  AGCCGGCGGG AGCAGCCGCC CACAGAGCCA GGCCCTCAGA GTGCCTCAGA
1251  GGTGGAGAAG ATCGCTCTGA ATTTGGAGGG GTGTGCCCTC AGCCAGGGCA
1301  GCCTCAGGAC GGGGACCCAG GAAGTGGGCG GTCAGGACCC TGGGGAGGCC
1351  TCCTGTCCCT GCTGCAGGGG CTGGGGCCTC CGGAGCTGCT GCGGGCTCCC
1401  CTCAGGCTCT GCTTCGTGAC CCGTGACCCA TGACCCACAG TGCTGGCCTC
1451  CTGTGGGGCC ACTATAGCAG CCACCAGAAG CCGCGAGGCC CTCAGGGAAG
1501  CCCAAGGCCT GCAGGAGCCT CCTGGCCTGG CTGTGTCTTC CCCACCCAGC
1551  TCTCCCCTGC GCCCCTGTCT TTGTAAATTG ACCCTTCTGG AGTGGGGGGC
1601  G
```

SEQ ID NO: 8 -- Human Opioid Growth Factor Receptor protein, from spliced cDNA version 1

```
MDDPDCDSTWEEDEEDAEDAEDEDCEDGEAAGARDADAGDEDEESEEPRAARPSSFQSRM   60
TGSRNWRATRDMCRYRHNYPDLVERDCNGDTPNLSFYRNEIRFLPNGCFIEDILQNWTDN  120
YDLLEDNHSYIQWLFPLREPGVNWHAKPLTLREVEVFKSSQEIQERLVRAYELMLGFYGI  180
RLEDRGTGTVGRAQNYQKRFQNLNWRSHNNLRITRILKSPCELSLEHFQAPLVRFFLEET  240
LVRRELPGVRQSALDYFMFAVRCRHQRRQLVHFAWEHFRPRCKFVWGPQDKLRRFKPSSL  300
PHPLEGSRKVEEEGSPGDPDHEASTQGRTCGPEHSKGGGRVDEGPQPRSVEPQDAGPLER  360
SQGDEAGGHGEDRPEPLSPKESKKRKLELSRREQPPTEPGPQSASEVEKIALNLEGCALS  420
QGSLRTGTQEVGGQDPGEASCPCCRGWGLRSCCGLPSGSAS                      461
```

SEQ ID NO: 9 -- Human OGFr cDNA, spliced version 4

```
   1  TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
  51  CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
 101  AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
 151  GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
 201  CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
 251  GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
 301  GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
 351  CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC
 401  TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
 451  CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
 501  GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
 551  TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
 601  GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
 651  CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
 701  TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
 751  ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
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 801  CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
 851  TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
 901  GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCGCATC CGCTCGAGGG
 951  CTCCAGGAAG GTGGAGGAGG AAGGAAGCCC CGGGGACCCC GACCACGAGG
1001  CCAGCACCCA GGGTCGGACC TGTGGGCCAG AGCATAGCAA GGGTGGGGGC
1051  AGGGTGGACG AGGGGCCCCA GCCACGGAGC GTGGAGCCCC AGGATGCGGG
1101  ACCCCTGGAG AGGAGCCAGG GGGATGAGGC AGGGGGCCAC GGGGAAGATA
1151  GGCCGGAGCC CTTAAGCCCC AAAGAGAGCA AGAAGAGGAA GCTGGAGCTG
1201  AGCCGGCGGG AGCAGCCGCC CACAGAGCCA GGCCCTCAGA GTGCCTCAGA
1251  GGTGGAGAAG ATCGCTCTGA ATTTGGAGGG GTGTGCCCTC AGCCAGGGCA
1301  GCCTCAGGAC GGGGACCCAG GAAGTGGGCG GTCAGGACCC TGGGGAGGCA
1351  GTGCAGCCCT GCCGCCAACC CCTGGGAGCC AGGGTGGCCG ACAAGGTGAG
1401  GAAGCGGAGG AAGGTGGATG AGGGTGCTGG GGACAGTGCT GCGGTGGCCA
1451  GTGGTGGTGC CCAGACCTTG GCCCTTGCCG GGTCCCCTGC CCCATCGGGG
1501  CACCCCAAGG CTGGACACAG TGAGAACGGG GTTGAGGAGG ACACAGAAGG
1551  TCGAACGGGG CCCAAAGAAG GTACCCCTGG GAGCCCATCG GAGACCCCAG
1601  GCCCCAGCCC AGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG
1651  GAGACCCCAG GCCCCCGCCC AGCAGGACCT GCAGGGGACG AGCCAGCCGA
1701  GAGCCCATCG GAGACCCCAG GCCTCCGCCC GGCAGGACCT GCAGGGGACG
1751  AGCCAGCCGA GACCCCATCG GAGACCCCAG GCCCCAGCCC GGCAGGACCT
1801  ACAAGGGATG AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC
1851  GGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG GAGACCCCAG
1901  GCCCCCGCCC GGCAGGACCT GCAGGGGACG AACCAGCCGA GAGCCCATCG
1951  GAGACCCCAG GCCCCAGCCC GGCAGGACCT ACAAGGGATG AGCCAGCCAA
2001  GGCGGGGGAG GCAGCAGAGT TGCAGGACGC AGAGGTGGAG TCTTCTGCCA
2051  AGTCTGGGAA GCCTTAAGGA AAGGAGTGCC CGTCGGCGTC TTGGTCCTCC
2101  TGTCCCTGCT GCAGGGGCTG GGGCCTCCGG AGCTGCTGCG GACTCCCCTC
2151  AGGCTCTGCT TCGTGACCCG TGACCCATGA CCCACAGTGC TGGCCTCCTG
2201  TGGGGCCACT ATAGCAGCCA CCAGAAGCCG CGAGGCCCTC AGGGAAGCCC
2251  AAGGCCTGCA GAAGCCTCCT GGCCTGGCTG TGTCTTCCCC ACCCAGCTCT
2301  CCCCTGCGCC CCTGTCTTTG TAAATTGACC CTTCTGGAGT GGGGGGCG
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SEQ ID NO: 10 -- Human OGFr, from spliced cDNA version 4

MDDPDCDSTWEEDEEDAEDAEDEDCEDGEAAGARDADAGDEDEESEEPRAARPSSFQSRM 60
TGSRNWRATRDMCRYRHNYPDLVERDCNGDTPNLSFYRNEIRFLPNGCFIEDILQNWTDN 120
YDLLEDNHSYIQWLFPLREPGVNWHAKPLTLREVEVFKSSQEIQERLVRAYELMLGFYGI 180
RLEDRGTGTVGRAQNYQKRFQNLNWRSHNNLRITRILKSPCELSLEHFQAPLVRFFLEET 240
LVRRELPGVRQSALDYFMFAVRCRHQRRQLVHFAWEHFRPRCKFVWGPQDKLRRFKPSSL 300
PHPLEGSRKVEEEGSPGDPDHEASTQGRTCGPEHSKGGGRVDEGPQPRSVEPQDAGPLER 360
SQGDEAGGHGEDRPEPLSPKESKKRKLELSRREQPPTEPGPQSASEVEKIALNLEGCALS 420
QGSLRTGTQEVGGQDPGEAVQPCRQPLGARVADKVRKRRKVDEGAGDSAAVASGGAQTLA 480
LAGSPAPSGHPKAGHSENGVEEDTEGRTGPKEGTPGSPSETPGPSPAGPAGDEPAESPSE 540
TPGPRPAGPAGDEPAESPSETPGLRPAGPAGDEPAETPSETPGPSPAGPTRDEPAESPSE 600
TPGPRPAGPAGDEPAESPSETPGPRPAGPAGDEPAESPSETPGPSPAGPTRDEPAKAGEA 660
AELQDAEVESSAKSGKP

SEQ ID NO: 11 -- Human OGFr cDNA, spliced version 7

```
      1  TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
     51  CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
    101  AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
    151  GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
    201  CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
    251  GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
    301  GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
    351  CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC
    401  TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
    451  CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
    501  GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
    551  TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
    601  GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
    651  CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
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 701  TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
 751  ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
 801  CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
 851  TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
 901  GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCCCATC CGCTCGAGGG
 951  CTCCAGGAAG GTGGAGGAGG AAGGAAGCCC CGGGGACCCC GACCACGAGG
1001  CCAGCACCCA GGGTCGGACC TGTGGGCCAG AGCATAGCAA GGGTGGGGGC
1051  AGGGTGGACG AGGGGCCCCA GCCACGGAGC GTGGAGCCCC AGGATGCGGG
1101  ACCCCTGGAG AGGAGCCAGG GGGATGAGGC AGGGGGCCAC GGGGAAGATA
1151  GGCCGGAGCC CTTAAGCCCC AAAGAGAGCA AGAAGAGGAA GCTGGAGCTG
1201  AGCCGGCGGG AGCAGCCGCC CACAGAGCCA GGCCCTCAGA GTGCCTCAGA
1251  GGTGGAGAAG ATCGCTCTGA ATTTGGAGGG GTGTGCCCTC AGCCAGGGCA
1301  GCCTCAGGAC GGGGACCCAG GAAGTGGGCG GTCAGGACCC TGGGGAGGCA
1351  GTGCAGCCCT GCCGCCAACC CCTGGGAGCC AGGGTGGCCG ACAAGGTGAG
1401  GAAGCGGAGG AAGGTGGATG AGGGTGCTGG GGACAGTGCT GCGGTGGCCA
1451  GTGGTGGTGC CCAGACCTTG GCCCTTGCCG GGTCCCCTGC CCCATCGGGG
1501  CACCCCAAGG CTGGACACAG TGAGAACGGG GTTGAGGAGG ACACAGAAGG
1551  TCGAACGGGG CCCAAAGAAG GTACCCCTGG GAGCCCATCG GAGACCCCAG
1601  GCCCCAGCCC AGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG
1651  GAGACCCCAG GCCCCCGCCC GGCAGGACCT GCAGGGGACG AGCCAGCCGA
1701  GAGCCCATCG GAGACCCCAG GCCCCAGCCC GGCAGGACCT ACAAGGGATG
1751  AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC GGCAGGACCT
1801  GCAGGGGACG AGCCAGCCGA GAGCCCATCG GAGACCCCAG GCCCCCGCCC
1851  GGCAGGACCT GCAGGGGACG AGCCAGCCGA GAGCCCATCG GAGACCCCAG
1901  GCCCCAGCCC GGCAGGACCT ACAAGGGATG AGCCAGCCAA GGCGGGGGAG
1951  GCAGCAGAGT TGCAGGACGC AGAGGTGGAG TCTTCTGCCA AGTCTGGGAA
2001  GCCTTAAGGA AAGGAGTGCC CGTCGGCGTC TTGGTCCTCC TGTCCCTGCT
2051  GCAGGGGCTG GGGCCTCCGG AGCTGCTGCG GGCTCCCCTC AGGCTCTGCT
2101  TCGTGACCCG TGACCCATGA CCCACAGTGC TGGCCTCCTG TGGGGCCACT
2151  ATAGCAGCCA CCAGAAGCCG CGAGGCCCTC AGGGAAGCCC AAGGCCTGCA
2201  GAAGCCTCCT GGCCTGGCTG TGTCTTCCCC ACCCAGCTCT CCCCTGCGCC
2251  CCTGTCTTTG TAAATTGACC CTTCTGGAGT GGGGGGCGG
```

SEQ ID NO: 12, Human OGFr, from spliced cDNA version 7

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MDDPDCDSTWEEDEEDAEDAEDEDCEDGEAAGARDADAGDEDEESEEPRAARPSSFQSRM   60
TGSRNWRATRDMCRYRHNYPDLVERDCNGDTPNLSFYRNEIRFLPNGCFIEDILQNWTDN  120
YDLLEDNHSYIQWLFPLREPGVNWHAKPLTLREVEVFKSSQEIQERLVRAYELMLGFYGI  180
RLEDRGTGTVGRAQNYQKRFQNLNWRSHNNLRITRILKSPCELSLEHFQAPLVRFFLEET  240
LVRRELPGVRQSALDYFMFAVRCRHQRRQLVHFAWEHFRPRCKFVWGPQDKLRRFKPSSL  300
PHPLEGSRKVEEEGSPGDPDHEASTQGRTCGPEHSKGGGRVDEGPQPRSVEPQDAGPLER  360
SQGDEAGGHGEDRPEPLSPKESKKRKLELSRREQPPTEPGPQSASEVEKIALNLEGCALS  420
QGSLRTGTQEVGGQDPGEAVQPCRQPLGARVADKVRKRRKVDEGAGDSAAVASGGAQTLA  480
LAGSPAPSGHPKAGHSENGVEEDTEGRTGPKEGTPGSPSETPGPSPAGPAGDEPAESPSE  540
TPGPRPAGPAGDEPAESPSETPGPSPAGPTRDEPAESPSETPGPRPAGPAGDEPAESPSE  600
TPGPRPAGPAGDEPAESPSETPGPSPAGPTRDEPAKAGEAAELQDAEVESSAKSGKP     657
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SEQ ID NO: 13 -- Human OGFr cDNA, spliced version 127

```
   1  TAGAATTCAG CGGCCGCTGA ATTCTAGCCG AGCATGGACG ACCCCGACTG
  51  CGACTCCACC TGGGAGGAGG ACGAGGAGGA TGCGGAGGAC GCGGAGGACG
 101  AGGACTGCGA GGACGGCGAG GCCGCCGGCG CGAGGGACGC GGACGCAGGG
 151  GACGAGGACG AGGAGTCGGA GGAGCCGCGG GCGGCGCGGC CCAGCTCGTT
 201  CCAGTCCAGA ATGACAGGGT CCAGAAACTG GCGAGCCACG AGGGACATGT
 251  GTAGGTATCG GCACAACTAT CCGGATCTGG TGGAACGAGA CTGCAATGGG
 301  GACACGCCAA ACCTGAGTTT CTACAGAAAT GAGATCCGCT TCCTGCCCAA
 351  CGGCTGTTTC ATTGAGGACA TTCTTCAGAA CTGGACGGAC AACTATGACC
 401  TCCTTGAGGA CAATCACTCC TACATCCAGT GGCTGTTTCC TCTGCGAGAA
 451  CCAGGAGTGA ACTGGCATGC CAAGCCCCTC ACGCTCAGGG AGGTCGAGGT
 501  GTTTAAAAGC TCCCAGGAGA TCCAGGAGCG GCTTGTCCGG GCCTACGAGC
 551  TCATGCTGGG CTTCTACGGG ATCCGGCTGG AGGACCGAGG CACGGGCACG
 601  GTGGGCCGAG CACAGAACTA CCAGAAGCGC TTCCAGAACC TGAACTGGCG
 651  CAGCCACAAC AACCTCCGCA TCACACGCAT CCTCAAGTCG CCGTGTGAGC
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701  TGAGCCTCGA GCACTTCCAG GCGCCACTGG TCCGCTTCTT CCTGGAGGAG
751  ACGCTGGTGC GGCGGGAGCT GCCGGGGGTG CGGCAGAGTG CCCTGGACTA
801  CTTCATGTTC GCCGTGCGCT GCCGACACCA GCGCCGCCAG CTGGTGCACT
851  TCGCCTGGGA GCACTTCCGG CCCCGCTGCA AGTTCGTCTG GGGGCCCCAA
901  GACAAGCTGC GGAGGTTCAA GCCCAGCTCT CTGCCGCATC CGCTCGAGGG
951  CTCCAGGAAG GTGGAGGAGG AAGGACCTGC AGGGGACGAG CCAGCCGAGA
1001 GCCCATCGGA GACCCCAGGC CCCAGCCCGG CAGGACCTAC AAGGGATGAG
1051 CCAGCCAAGG CGGGGGAGGC AGAAGCCTGC TGCCTGGCTG TGTCTTCCCA
1101 CCCAGCTCTC CCCTGCGCCC CTGTCTTTGT TAATCGACCC TTCTGGAGCG
1151 GGGGGCGGCG GGCAGGGCTT GCCTTTCTTA GTCTGATGCC AAGCAAGGCC
1201 TTTTCTGAAT AAATTCATTT GACTTTCGAA AA
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SEQ ID NO: 14 -- Human OGFr, from spliced cDNA version 127

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MDDPDCDSTWEEDEEDAEDAEDEDCEDGEAAGARDADAGDEDEESEEPRAARPSSFQSRM   60
TGSRNWRATRDMCRYRHNYPDLVERDCNGDTPNLSFYRNEIRFLPNGCFIEDILQNWTDN  120
YDLLEDNHSYIQWLFPLREPGVNWHAKPLTLREVEVFKSSQEIQERLVRAYELMLGFYGI  180
RLEDRGTGTVGRAQNYQKRFQNLNWRSHNNLRITRILKSPCELSLEHFQAPLVRFFLEET  240
LVRRELPGVRQSALDYFMFAVRCRHQRRQLVHFAWEHFRPRCKFVWGPQDKLRRFKPSSL  300
PHPLEGSRKVEEEGPAGDEPAESPSETPGPSPAGPTRDEPAKAGEAEACCLAVSSHPALP  360
CAPVFVNRPFWSGGRRAGLAFLSLMPSKAFSE                              392
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WE CLAIM:

1. An isolated nucleic acid molecule comprising any of SEQ ID NOs: 1, 4-5, 7, 9, 11 or 13, or a fragment thereof.

2. An isolated nucleic acid molecule substantially homologous to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 or 13.

3. An isolated nucleic acid molecule, the complement sequence of which hybridize under stringent conditions to any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

4. An isolated nucleic acid molecule comprising an antisense sequence of any of SEQ ID NOs: 1, 4-5, 7, 9, 11 and 13.

5. An expression vector comprising any of the isolated nucleic acid molecules of claims 1-4.

6. A cell, transformed with the expression vector of claim 5.

7. A method of producing an OGFr protein or a fragment thereof, comprising transforming a host cell with an expression vector, wherein said expression vector encodes said OGFr protein or a fragment thereof, expressing said OGFr protein or said fragment thereof in the cell and recovering said protein or said fragment thereof.

8. The method of claim 7, wherein said OGFr is encoded by any of SEQ ID NOs: 1, 4-5, 7, 9, 11 or 13.

9. An isolated protein consisting any of SEQ ID NOs: 2, 6, 8, 10, 12 and 14.

10. The isolated protein of claim 9, wherein said protein is made recombinantly.

11. A functional derivative of any of SEQ ID NO: 2, 6, 8, 10, 12 and 14.

12. An antibody directed against an OGFr protein consisting of any of SEQ ID NOs: 2, 6, 8, 10, 12 and 14.

13. The antibody of claim 10, wherein said antibody is a monoclonal antibody.

14. A pharmaceutical composition comprising the isolated nucleic acid molecule of claim 1 and a pharmaceutically acceptable carrier.

15. A pharmaceutical composition comprising the isolated nucleic acid molecule of claim 4 and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising the expression vector of claim 5 and a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising the cell of claim 6 and a pharmaceutically acceptable carrier.

18. A pharmaceutical composition comprising the isolated protein of claim 9 and a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising the functional derivative of claim 11 and a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising the antibody of claim 12 and a pharmaceutically acceptable carrier.

21. A method of detecting the expression of an OGF receptor in a tissue of a subject, comprising contacting a sample of said tissue with a nucleic acid sequence encoding said OGFr or a portion thereof and determining the level of the mRNA encoding said OGFr.

22. A method of detecting the level of an OGF receptor in a tissue of a subject, comprising contacting a sample of said tissue with an antibody specific for said OGFr, detecting and quantitating the complexes formed between said OGFr and said antibody.

23. A method of inhibiting growth of cells *in vitro* comprising introducing to said cells an effective amount of nucleic acid molecules coding for an OGFr or a functional derivative thereof.

24. A method of promoting growth of cells *in vitro* comprising introducing to said cells an effective amount of an OGFr antisense molecule.

25. A method of promoting growth of cells *in vitro* comprising introducing to said cells an effective amount of an antibody directed against an OGFr expressed in such cells.

26. A method of treating cancer in a patient comprising enhancing the function of the OGF ligand-receptor system in the cancerous cells of said patient.

27. A method of treating cancer in a patient comprising administering to said patient, an effective amount of a nucleic acid molecule coding for an OGFr or a functional derivative thereof.

28. The method of claim 27, wherein said cancer is selected from the group consisting of a cancer of neural tissues, prostate cancer, breast cancer, head and neck cancer, and gastrointestinal cancer.

29. The method of claim 28, wherein said gastrointestinal cancer is selected from the group consisting of a pharyngeal, esophageal, stomach, small and large intestine, liver, rectal, colon, pancreatic, biliary tract cancer.

30. The method of claim 27, wherein said cancer is characterized by a deficiency of OGF receptors.

31. The method of claim 27, further comprising administering OGF to said subject.

32. A method of treating a subject with a cancer characterized by a deficiency of OGF receptors, comprising determining the deficiency of OGF receptors on the cancerous cells in said subject, and administering to the subject an effective amount of a nucleic acid molecule coding for an OGFr or a functional derivative thereof.

33. The method of claim 33, further comprising adminisitering OGF to said subject.

34. A method of promoting growth of cells in a subject in need thereof comprising interfering with the function of the OGF ligand-receptor system in said subject.

35. The method of claim 34, wherein said subject suffers a tissue wound.

36. The method of claim 34, comprising administering to said subject, an effective amount of an OGFr antisense molecule.

37. The method of claim 34, comprising administering to said subject, an effective amount of an antibody against an OGFr.

ABSTRACT

This invention relates to novel nucleic acid molecules coding for opioid growth factor receptors. In particular, the present invention provides isolated nucleic acid molecules coding for human and rat OGF receptors. Antisense molecules, expression vectors and host cells, isolated proteins encoded by such nucleic acid molecules, antibodies directed against such proteins, as well as pharmaceutical compositions derived therefrom are also included. The invention further provides methods of modulating cell growth by using the isolated nucleic acid molecules, the antisense molecules and the antibodies directed against the encoded proteins.